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ORIGINAL ARTICLE

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Anti-Ro52/TRIM21 autoantibodies predict Sjögren's syndrome in patients with primary biliary cholangitis

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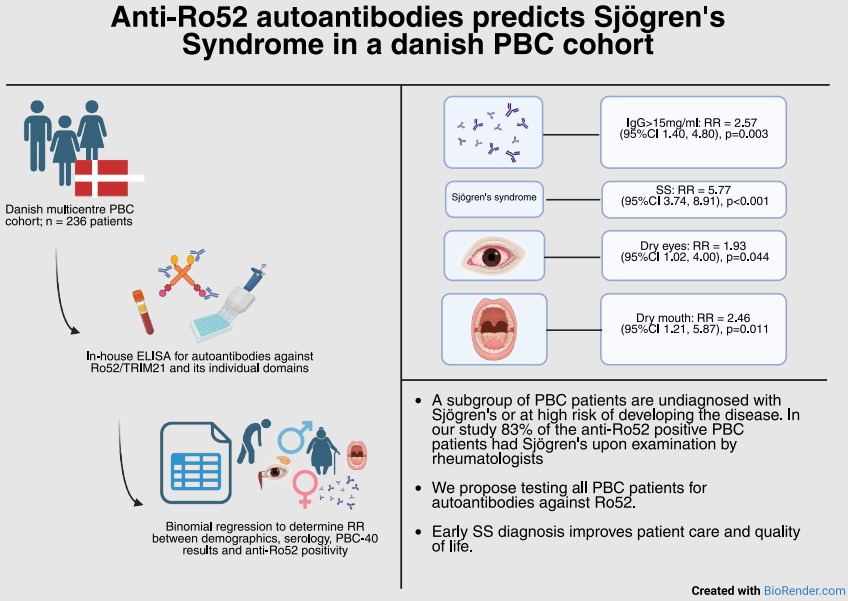
Graphical Abstract



Abstract

Background and aims: Sjögren's syndrome is a common comorbidity in patients with primary biliary cholangitis (PBC). Anti-Ro52 autoantibodies against the protein TRIM21 are often seen in Sjögren's syndrome. TRIM21 consists of four domains (PRY/SPRY, Coiled-Coil, B-box, and RING domain), each with a specific function. We hypothesized that patients with PBC and concomitant autoantibodies against TRIM21 had a higher risk of developing Sjögren's syndrome.

Abbreviations: AIH, Autoimmune Hepatitis; ALP, alkaline phosphatase; AMAs, Antimitochondrial autoantibodies; Anti-Ro52, Anti-Ro52/TRIM21 autoantibodies; EASL, the European Association for the Study of the Liver; HRQoL, health-related quality of life; IQR, Interquartile range; PBC, Primary Biliary Cholangitis; RR, Risk rate; SS, Sjögren's syndrome; TE, transient elastography.

Methods: Using ELISA, we analyzed plasma from 236 Danish patients with PBC. Binomial regression assessed the risk rates between demographics, serologic variables, PBC-40 results, and autoantibody positivity.

Results: Forty patients (16.9%) tested positive for anti-Ro52 autoantibodies, with 23 (9.7%) samples positive against Coiled-Coil, 12 against PRY/SPRY (5.1%), and 10 against RING (4.2%). Increased IgG plasma levels, reports of *dry mouth*, and a diagnosis of Sjögren's syndrome increased the risk of both anti-Ro52 and all domain autoantibodies. In the anti-Ro52 positive subgroup, no less than 14 undiagnosed patients met the criteria for Sjögren's syndrome.

Conclusions: We observed a 16.9% positivity of anti-Ro52 autoantibodies and an association between having these autoantibodies and elevated IgG, the symptom *dry mouth*, and having Sjögren's syndrome. Furthermore, we found a sizable undiagnosed group of Sjögren's patients in the anti-Ro52 positive subgroup. Our results suggest that Sjögren's patients are underdiagnosed in patients with PBC. We propose that patients with PBC be tested for anti-Ro52 autoantibodies and, if positive, referred for rheumatological examination.

KEYWORDS
autoantibodies, autoimmune disease, primary biliary cholangitis, Sjögren's syndrome, TRIM21

INTRODUCTION

One in 10 people is living with an autoimmune disease, and that number is rising.[1] Having one autoimmune disease increases the risk of developing additional autoimmune diseases.[1] Therefore, clinicians must be attentive toward symptomatology originating outside their specialty and screen for autoimmune comorbidities.

Primary biliary cholangitis is an autoimmune liver disease affecting the intrahepatic small bile ducts. Patients suffering from PBC develop persistent cholestasis and cholangitis, resulting in biliary cirrhosis and, without treatment, end-stage liver disease.[2] PBC is characterized by antimitochondrial autoantibodies (AMAs) and infiltration of mononuclear cells into the bile duct.[2] Apart from AMA, other autoantibodies such as anti-dsDNA, anti-centromere, and anti-Ro52/TRIM21 autoantibodies can be detected in PBC.[3,4] Patients with PBC often experience other autoimmune diseases, such as autoimmune hepatitis (AIH). In cases of PBC-AIH-overlap syndrome, there is a greater likelihood of requiring liver transplantation and higher mortality rates.[5]

Sjögren's syndrome (SS) is an autoimmune disease that causes sicca symptoms, fatigue, and arthralgia, among others.[6] The patients primarily experience dryness of the eyes and mouth, but also dry skin, dry mucosal membranes, dry cough, and constipation.[6] Patients with SS have an increased risk of lymphoma; thus, they should be followed according to current guidelines.[7,8] Anti–Sjögren's-syndrome-related antigen A autoantibodies (anti-SSA-antibodies) are present in around 80% of the patients and are a cornerstone in the research classification criteria.[7,9] Anti-SSA target Ro proteins, mainly Ro52 and Ro60. Assays for detecting anti-SSA often simultaneously assess anti-Ro52 autoantibodies (herein called anti-Ro52) and anti-Ro60 autoantibodies.[10]

Anti-Ro52 is most commonly seen in SS, where it is reported in around 80%–100% of the patients, but it may also be present in other autoimmune diseases.[11,12] The antibodies target TRIM21– an intracellular protein vital in our antiviral defense.[13] TRIM21 consists of 4 domains: PRY/SPRY, Coiled-Coil, B-box, and RING, and patients may exhibit antibodies against different parts of the protein (Figure 1).[13,14] The presence of anti-Ro52, without anti-Ro60, has been associated with a poorer prognosis in both autoimmune rheumatic disease and PBC.[15,16]

We hypothesized that testing for anti-Ro52 can increase the likelihood of detecting SS in patients with PBC. We aimed to investigate this by first examining a large well-characterized cohort of PBC patients for autoantibodies against TRIM21 and three of its individual domains. Secondly, we investigated the correlations between the presence of these autoantibodies and demographics, comorbidities, and quality of life as assessed by the PBC-40 questionnaire.

MATERIALS AND METHODS

Subjects

The study was conducted on a cohort of patients with PBC (n = 236) from the central and northern Denmark region, earlier described in detail.[17] Patients were either included at the Department of Hepatology and Gastroenterology at Aarhus University Hospital or the Department of Hepatology and Gastroenterology at Aalborg University Hospital. Inclusion was performed between 2016 and 2020. All subjects were either newly diagnosed

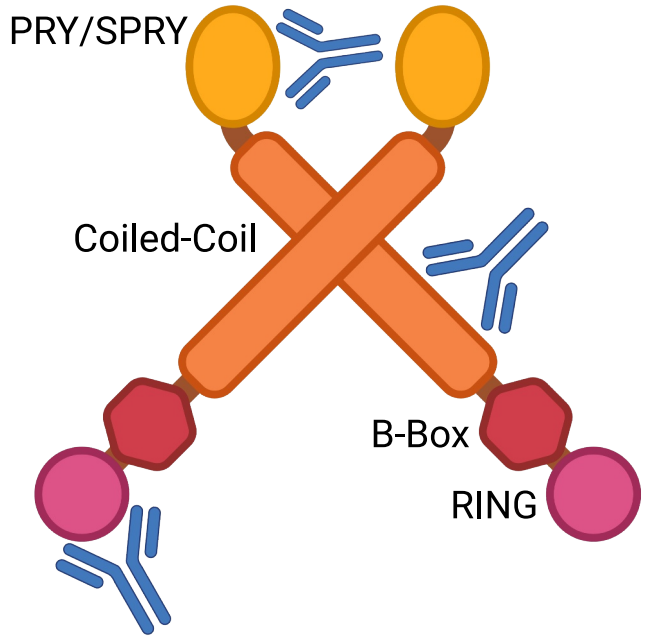


FIGURE 1 Schematic figure of the TRIM21 protein and its four individual domains. PRY/SPRY (yellow), Coiled-Coil (orange), B-box (red), and RING (pink). The protein is shown as a dimer. Immunoglobulin G autoantibodies (blue) against three domains have been reported.[14] Made with Biorender.com.

with PBC (n = 79) (incident, i.e. before treatment initiation with ursodeoxycholic acid) or had known disease (n = 157) (prevalent). The diagnosis of PBC (n = 205) and PBC-AIH (n = 31) was confirmed according to the guidelines from the European Association for the Study of Liver (EASL).[18] In statistical analysis, the cohort was divided into a PBC-only group without AIH and a PBC-AIH overlap group. Patients of both groups experience other comorbidities, as described in the results section. The SS diagnosis was obtained by chart review and validated according to the 2016 ACR-EULAR criteria for primary Sjögren's syndrome.[7] Plasma was collected at the time of inclusion and stored at−80°C until use. All further inclusion and data collection followed the standard inclusion protocol.

PBC-40

PBC-40 is a validated questionnaire to assess health-related quality of life (HRQoL) in patients with PBC. It consists of 40 questions within six domains – general symptoms, itch, fatigue, cognitive, social, and emotional.[19] Each question is scored by the patient on a five-point scale (1 = never to 5 = always) or as not applicable depending on the question (0 = not applicable) – the higher the domain score, the greater the influence of the disease on the quality of life. The scores of each domain can be divided into severity categories of none, mild, moderate, or severe (Appendix Table A1).[20] Scores within the none and mild categories are comparable to scores of the general population.[20,21] The questionnaire was filled out by the patients at the time of inclusion. If a domain was incomplete, the recommended approach by the UK-PBC was followed: If > 50% of the answers to a domain are missing, the domain should be discarded. If <50% of the answers are missing, the median value for the remaining items in the domain should be used for the missing items.[22] The scores of the entire domain and the corresponding severity (none to severe) were used for analysis. In addition, the two questions regarding dryness of the mouth and eyes from the general symptom's domain were also analyzed separately, where the score 1 was used as no dryness, and the scores 2–5 were used as dryness.

Detection of autoantibodies

An indirect ELISA assay was used to assess autoantibodies against the full-length TRIM21 protein and the three domains RING, Coiled-Coil, and PRY/SPRY.[14] A choice was made not to analyze anti-B-box antibodies, as we have not detected these earlier.[14] All samples were run in duplicates. A blank well containing dilution buffer instead of patient sample was run on every plate, and the value was subtracted from the remaining samples before calculations. The optical density was measured at 450 nm with a 540 nm wavelength correction using a Thermo Scientific Multiskan GO reader.

Cut-off values

Samples from healthy controls (HC) (n = 40) were analyzed in duplicate on the full-length and the three domain ELISAs. The mean OD value + 3SD for HC in each assay was calculated. Three HC samples, which simulate the mean OD + 3SD value of the HC group, were selected for each ELISA assay and run on every plate throughout the analysis. The individual plate HC mean OD + 3SD was used as the cut-off value for that particular plate.

Antibody positivity

All samples above the individual plate cut-off are reported as positive. Only full-length ELISA-positive samples were run on the domain ELISAs. By default, all samples negative on the full-length ELISA were registered as negative on the domain ELISAs.

Ethics

Samples were obtained after informed written patient consent. The study was approved by the Local Ethics Committee (case no 1-10-72-149-16) and reported to clinicaltrials.gov (NCT02924701 and NCT02931513). Finally, the study was conducted in accordance with the Declarations of Helsinki.

Data analysis

Categorical variables are presented as numbers and percentages, and continuous variables as medians with interquartile ranges (IQR). Associations were tested using the chi-squared, Fisher's exact, and Wilcoxon rank sum tests as appropriate. A univariate regression model calculated risk rates (RR) with corresponding 95% confidence intervals (95% CI) and *p*-values. Missing values were blinded from the analysis. For sensitivity analysis of the univariate model, autoimmune hepatitis (AIH) and Sjögren's syndrome (SS) were excluded separately, and all regressions were redone. All statistical calculations, tables, and figures were performed in R (version 4.3.1).

RESULTS

Demographics of the PBC-only group and the PBC-AIH overlap group

The PBC-only group (n = 205) was predominantly females (88%), with a median age of 62.0 years and a body mass index of 25.3 kg/m^2 (Table 1). The patients had elevated alkaline phosphatase (ALP) and IgM levels. The majority of patients had only mild fibrosis using FibroScan with transient elastography (TE) of 5.6 kPa. However, 20 subjects (8.7%) had a TE > 16.9 kPa, indicating cirrhosis. In the PBC-only group, 33.8% were diagnosed with one or more comorbidities registered at the time of inclusion. The three most common

TABLE 1 Cohort demographics.

	PBC-only, n = 205[a]	PBC-AIH overlap, n = 31[a]	p-value[b]
Female	181 (88%)	26 (84%)	0.6
Age, years	62.0 (53.0, 69.0)	53.0 (49.0, 64.5)	0.021
BMI (kg/m2)	25.3 (22.8, 29.2)	27.6 (23.1, 30.8)	0.2
TE (kPa)	5.6 (4.7, 8.2)	7.5 (5.3, 11.2)	0.050
AMA positive at diagnosis	155 (79%)	24 (83%)	0.6
Biochemical			
ALAT (U/L) ref: female <45, male <70	40 (25, 66)	39 (23, 53)	0.5
Bilirubin (µmol/L) ref: <25	9 (6, 12)	10 (8, 17)	0.047
ALP (U/L) ref: <105	177 (127, 297)	107 (82, 195)	<0.001
Albumin (g/L) ref: 36–45	37 (35, 39)	38 (37, 40)	0.2
IgG (g/L) ref: <15.0	12.9 (10.7, 15.4)	13.8 (11.7, 17.0)	0.10
IgM (g/L) ref: <2.08	2.69 (1.71, 4.00)	2.52 (1.26, 3.65)	0.2
sCD163 (mg/L) ref: <3.86	3.96 (2.85, 5.27)	3.23 (2.77, 4.59)	0.2
Comorbidities			
Diabetes	16 (7.8%)	7 (22.6%)	0.019
Osteoporosis	32 (15.7%)	6 (19.4%)	0.6
Sjögren's syndrome	6 (3.0%)	1 (3.2%)	>0.9
Myxedema	18 (8.8%)	6 (19.4%)	0.10
Scleroderma	5 (2.5%)	0 (0.0%)	>0.9
Multiple comorbidities			0.056
0	135 (66.2%)	14 (45.2%)	
1	62 (30.4%)	15 (48.4%)	
2	6 (2.9%)	1 (3.2%)	
3	1 (0.5%)	1 (3.2%)	

Note: Table 1 showing demographics of the PBC cohort divided into PBC-only (without AIH) and PBC-AIH overlap syndrome.

Abbreviations: AIH: Autoimmune hepatitis; ALAT: Alanine aminotransferase; ALP: Alkaline phosphatase; BMI: Body mass index; IgG: Immunoglobulin G; IgM: Immunoglobulin M; PBC: Primary biliary cholangitis; TE: Transient Elastography.

[a]*n* (%); Median (IQR).

[b]Pearson's Chi-squared test; Fisher exact test; Wilcoxon rank sum test.

comorbidities were osteoporosis (15.7%), myxedema (8.8%), and diabetes (7.8%) (both type 1 and 2). Only 3.4% of the patients in the PBC-only group were diagnosed with two or more comorbidities.

The patients of the PBC-AIH overlap group (n = 31) were younger (53.0 vs. 62.0 years, $p = 0.021$). They generally showed signs of more progressive disease with a higher median TE of 7.5 versus 5.6 kPa in the PBC-only group ($p = 0.05$), higher bilirubin ($p = 0.047$), and lower ALP ($p < 0.001$). More patients in the PBC-AIH overlap group had diabetes than in the PBC-only group (22.6 vs. 7.8%, $p = 0.019$). The PBC-AIH overlap group showed a tendency toward having more comorbidities (54.8% vs. 33.8%, $p = 0.056$).

PBC-40 results

The median domain scores from the PBC-40 questionnaire are shown in Table 2. The PBC-AIH overlap group did not differ significantly in their PBC-40 score from the PBC-only group (Table 2). Therefore, the severity analysis within the domains was performed using the entire cohort. The HRQoL of the patients in the cohort was most affected by fatigue, as 45% (n = 98) of the patients had a fatigue score in the moderate to severe category (Figure 2). Thirty-seven per cent of patients were affected emotionally by their PBC, and around one in four were affected cognitively (28.2%), socially (22.3%) or by general symptoms (24.3%) (Figure 2). The domain which affected the HRQoL in the cohort the least was itching (12.7%) (Figure 2).

Anti-Ro52 autoantibodies are common in patients suffering from primary biliary cholangitis

We detected IgG autoantibodies toward the full-length TRIM21 protein in the plasma from 40 (16.9%) of the PBC patients. We proceeded to examine which part of the TRIM21 protein these autoantibodies bound to. Twenty-three samples (9.7%) were positive toward the Coiled-Coil domain, 12 (5.1%) toward the PRY/SPRY domain and 10 (4.2%) toward the RING domain. There was no difference in anti-Ro52 positivity between the PBC-only and the PBC-

TABLE 2 PBC-40.

	PBC-only, n = 205[a]	PBC-AIH overlap, n = 31[a]	p-value[b]
General symptoms domain	14 (10, 19)	14 (10, 15)	0.4
Itch domain	3 (3, 6)	3.0 (2.3, 4.0)	0.2
Fatigue domain	27 (18, 38)	24 (19, 40)	0.8
Cognitive domain	10 (6, 17)	10.0 (6, 16.0)	>0.9
Emotional domain	6 (4, 9)	6 (4, 7)	0.6
Social domain	19 (13, 28)	19 (15, 27)	>0.9

Note: Table 2: PBC-40 domain scores in medians with interquartile range for the PBC-only and PBC-AIH group respectively.
Abbreviation: AIH: Autoimmune hepatitis; PBC: Primary biliary cholangitis.
[a]Median (IQR).
[b]Wilcoxon rank sum test.

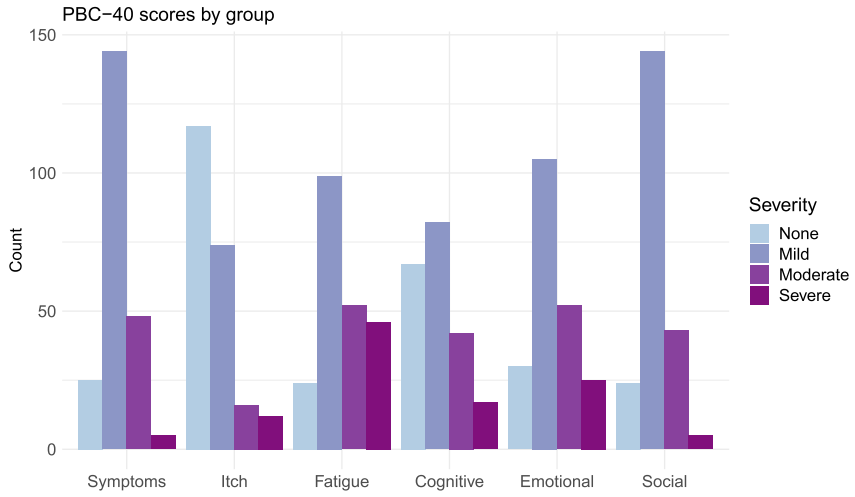


FIGURE 2 Bar chart of PBC-40 scores divided into severity scores for each domain. Severity scores are defined in the Appendix Table A1.

AIH overlap group, nor were there any differences between the incident and the prevalent group (data not shown).

Unadjusted regression analysis

We examined if clinical characteristics were associated with the presence of autoantibodies toward full-length TRIM21 or any of its domains. High levels of serum IgG (>15 mg/mL) and a known diagnosis of Sjögren's syndrome were associated with an increased risk of antibodies against the full-length TRIM21 protein and all three analyzed TRIM21 domains (Table 3). When analyzing the PBC-40 results, the symptom *dry eyes* from the symptom's domain was associated with a twofold higher risk of having anti-Ro52 and anti-PRY/SPRY autoantibodies. Similarly, *dry mouth* was associated with a higher risk of having autoantibodies against the Coiled-Coil domain, the PRY/SPRY domain, and the full-length TRIM21 protein (RR = 2.5, 95%CI 1.21, 5.87, $p = 0.011$). Looking at the clinical spectrum of PBC, a PBC-40 domain score in the moderate or severe category was not associated with having any of the measured autoantibodies.

When the PBC-AIH overlap group was excluded for sensitivity analysis, the association between increased IgG, SS, and a higher risk of all autoantibodies remained (Appendix - Table A2). The association between *dry mouth* and the anti-Ro52 and anti-Coiled-Coil, as well as between dry eyes and anti-PRY/SPRY, remained.

When the patients with SS were excluded for sensitivity analysis (Appendix - Table A3), the association between increased IgG and the risk of anti-Ro52, anti-Coiled-Coil, and anti-PRY/SPRY antibodies remained. *Dry mouth* showed a strong trend for association with a twice as high risk of having the anti-Ro52 (RR = 2.07 (0.99–5.01), $p = 0.073$). This was also shown in a post hoc analysis where the PBC and PBC-SS groups significantly differed within the PBC-40 symptoms domain (PBC median 14.0 (10.0, 18.0) versus PBC-SS 18.0 (16.0, 20.0) $p = 0.032$). All other PBC-40 domains were comparable within the two groups (data not shown).

Clinical relevance

Of the 40 patients with anti-Ro52, six patients were diagnosed with SS before being included in the cohort. To further investigate the

TABLE 3 Unadjusted risk rates (RR).

		TRIM21		RING		Coiled-Coil		PRY/SPRY	
Characteristics	**N**	**RR (95% CI)**	***p*-value**	**RR (95% CI)**	***p*-value**	**RR (95% CI)**	***p*-value**	**RR (95% CI)**	***p*-value**
AMA positive at diagnosis	226	1.40 (0.68, 3.54)	0.4	1.84 (0.34, 33.9)	0.5	1.58 (0.56, 6.52)	0.4	0.61 (0.18, 2.76)	0.5
IgG >15 mg/mL	217	2.57 (1.40, 4.80)	0.003	6.86 (1.63, 46.0)	0.008	3.43 (1.49, 8.40)	0.004	8.01 (2.00, 52.8)	0.003
IgM >2.08 mg/mL	231	1.16 (0.65, 2.22)	0.6	0.87 (0.26, 3.34)	0.8	2.10 (0.87, 6.17)	0.10	1.16 (0.38, 4.26)	0.8
Fibro scan >9.6 kPa	229	0.97 (0.44, 1.85)	>0.9	0.97 (0.15, 3.71)	>0.9	1.08 (0.37, 2.54)	0.9	0.77 (0.12, 2.82)	0.7
sCD163 > 3.86 mg/L	231	1.15 (0.65, 2.05)	0.6	1.57 (0.46, 5.99)	0.5	1.36 (0.62, 3.06)	0.4	1.46 (0.48, 4.82)	0.5
AIH	236	0.73 (0.23, 1.68)	0.5	1.65 (0.26, 6.24)	0.5	0.99 (0.24, 2.68)	>0.9	1.32 (0.21, 4.73)	0.7
Diabetes	235	0.77 (0.20, 1.91)	0.6	–	–	–	–	–	–
Osteoporosis	235	1.13 (0.49, 2.22)	0.7	0.58 (0.03, 2.94)	0.6	1.94 (0.74, 4.40)	0.2	1.15 (0.18, 4.26)	0.9
Sjögren's syndrome	235	5.77 (3.74, 8.90)	<0.001	14.02 (4.55, 43.15)	0.002	9.09 (4.77, 17.32)	<0.001	16.36 (6.42, 41.68)	<0.001
Myxedema	235	1.00 (0.32, 2.25)	>0.9	0.98 (0.05, 4.88)	>0.9	0.42 (0.02, 1.86)	0.3	–	–
Scleroderma	235	1.21 (0.08, 4.03)	0.8	–	–	–	–	–	–
Dry eyes	221	1.93 (1.02, 4.00)	0.044	2.85 (0.74, 18.6)	0.14	1.90 (0.82, 5.13)	0.14	7.84 (1.57, 142)	0.008
Dry mouth	222	2.46 (1.21, 5.87)	0.011	2.30 (0.59, 15.0)	0.2	2.59 (1.01, 8.72)	0.049	6.32 (1.26, 114)	0.021
Symptoms domain	236	0.61 (0.24, 1.26)	0.2	0.38 (0.02, 1.97)	0.3	0.52 (0.13, 1.44)	0.2	0.69 (0.11, 2.52)	0.6
Fatigue domain	236	0.84 (0.46, 1.50)	0.6	0.35 (0.05, 1.37)	0.14	0.50 (0.18, 1.15)	0.10	0.28 (0.04, 1.04)	0.058
Itch domain	236	0.39 (0.07, 1.18)	0.11	–	–	0.34 (0.02, 1.51)	0.2	–	–
Social domain	236	0.56 (0.20, 1.22)	0.2	0.44 (0.02, 2.23)	0.4	0.37 (0.06, 1.21)	0.11	0.36 (0.02, 1.76)	0.2
Emotional domain	236	0.78 (0.39, 1.43)	0.4	0.23 (0.01, 1.19)	0.086	0.57 (0.20, 1.38)	0.2	0.19 (0.01, 0.94)	0.040
Cognitive domain	236	1.14 (0.58, 2.06)	0.7	1.29 (0.28, 4.47)	0.7	1.06 (0.40, 2.42)	0.9	1.00 (0.23, 3.23)	>0.9

Note: Table 3: Unadjusted relative risk for antibody positivity with different categorical variables as exposure for the entire cohort. For all PBC-40 domains, patients are divided into two groups with no symptoms (none or mild severity category) or symptoms (moderate or severe category). All risks are calculated using binomial regression.

Abbreviations: AMA: Anti-Mitochondrial Antibodies; AIH: Autoimmune Hepatitis; IgG: Immunoglobulin G; IgM: Immunoglobulin M; RR: Relative Risk.

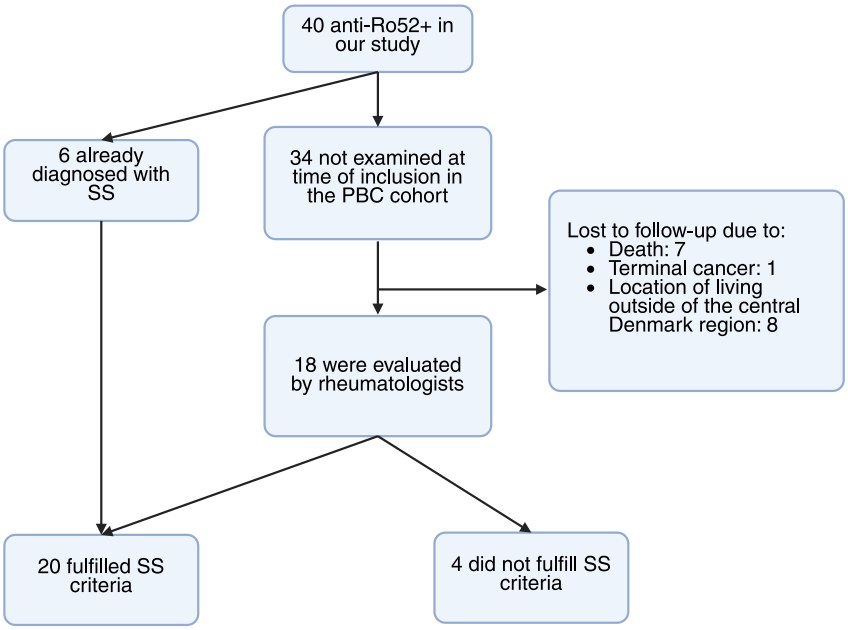


FIGURE 3 Flow chart of the anti-Ro52 positive patients of the cohort who were evaluated for Sjögren's syndrome. Made with Biorender.com.

prevalence of SS within the anti-Ro52-positive group, the remaining anti-Ro52-positive patients, who were available for examination ($n = 18$), were examined by a consultant rheumatologist. A flow chart of examined patients and patients who were lost to follow-up is shown in Figure 3. All results of the examination, that is, the Schirmer's test, the salivary test, and anti-SSA antibody positivity, were evaluated by a rheumatology fellow using the 2016 ACR-EULAR SS criteria.[7] A total of 24 of the 40 anti-Ro52 positive patients were available for examination, and of those, 20 (83%) fulfilled the SS classification criteria. Of the 20 PBC-SS patients, 17 (85%) were anti-SSA positive, and five out of 20 (25%) were anti-SSB positive. This suggests that our in-house anti-Ro52 assay is more sensitive for identifying SS patients within this PBC cohort. Anti-Ro52 is known to be associated with interstitial lung disease and myositis; however, none of the 24 examined patients were diagnosed with either of these conditions.

DISCUSSION

To our knowledge, this is the first analysis of autoantibodies against the individual domains of TRIM21 in a large well-characterized cohort of patients with PBC. Our main findings are that autoantibodies against the full-length TRIM21 protein independently predicted SS within this PBC cohort. We observed a large proportion of anti-Ro52-positive patients with PBC who, when examined by trained rheumatologists, actually had SS. This suggests that all patients with PBC should be screened for anti-Ro52, as it is a strong risk factor for SS in this PBC cohort and potentially minimizes diagnostic delay.

We showed that 16.9% of the PBC cohort have autoantibodies against the full-length protein. This is comparable to the recent study by Nguyen et al. but less than that reported in other studies.[4,23,24] It is well established that epitope spreading happens over time and that developing one autoimmune disease increases the risk of developing another.[1] Thus, a cohort consisting of 1/3 of incident patients is expected to exhibit fewer autoantibodies and autoimmune comorbidities than one consisting of patients diagnosed for years.[25,26] However, we did not find an increase in antibody positivity between our incident and prevalent group. Another explanation could be that different assays use different cut-offs. In this study, we employed a cut-off based on the values of 40 HC. This cut-off mimics the expected value in the healthy background population. As anti-Ro52 is present in up to 1% of the general population, our cut-off might be higher than that of commercial assays. However, we have earlier reported that our assay has a specificity and sensitivity of >0.90, comparable to commercial assays.[14] Finally, other methods such as the line blot used in studies by Granito et al. and Zhao et al. have a lower specificity than an ELISA, which could explain the higher percentage of positive patients reported in these papers.[23,24]

We showed that 9.7% of the PBC cohort were positive toward the Coiled-Coil domain, 5.1% toward the PRY/SPRY domain, and 4.2% toward the RING domain. The positivity rates toward each domain are similar to those previously shown in SS and SLE.[14] The three domains exhibit different functions in the functional TRIM21 protein.[27] The RING domain's primary role is the ubiquitination of antigens before degradation in the proteasome. The Coiled-Coil domain is a supercoiled structure that dimerizes TRIM21 monomers.[27] The PRY/SPRY domain is the Fc-binding site for a pathogen-associated immunoglobulin.[27] Autoantibodies against different

domains could lead to different immunological malfunctions and, thereby, different phenotypes in anti-Ro52-positive patients. In our study, there was a higher risk of having the anti-PRY/SPRY antibodies when exhibiting the symptom of *dry eyes*. However, this correlation disappeared when SS patients were excluded for sensitivity analysis.

Regression analysis showed that anti-Ro52 can predict SS within our PBC cohort. We did a post hoc analysis on the 40 patients with anti-Ro52. Of those, six were diagnosed with SS before inclusion, and of those afterward examined, 14 (58.3%) were diagnosed (Figure 3). To our knowledge, this is the first study showing that the presence of anti-Ro52 in a PBC patient can predict SS. The results contrast a recent study by Zhao et al. showing that anti-Ro60, but not anti-Ro52, is associated with SS in a PBC cohort.[24] The baseline prevalence of SS in this PBC cohort is lower than previously reported, with earlier studies suggesting a prevalence up to 35%.[28] This may have influenced our findings, and validation in independent cohorts is warranted.

Anti-Ro52 is also associated with other autoimmune diseases. In the anti-Ro52 positive group, two patients had rheumatoid arthritis, one had systemic lupus erythematosus, one had antiphospholipid syndrome, and two had scleroderma. However, the authors do not believe these concomitant autoimmune diseases fully explain the presence of anti-Ro52. Therefore, we propose that all PBC patients be tested for anti-Ro52 and, if positive, referred for rheumatological evaluation. This could reduce the diagnostic delay of SS, which was evident in our cohort and is well documented in the literature, thereby allowing patients to receive appropriate care earlier.[29] Furthermore, particular attention should be paid to the development of sicca symptoms in this high-risk subgroup of PBC patients.

We found that an increased level of IgG >15 mg/mL was associated with having anti-Ro52. Primary biliary cholangitis is associated with elevated IgM, whereas increased IgG is commonly observed in patients suffering from inflammatory diseases, including SS and AIH.[30] The association remained after a sensitivity analysis, excluding the PBC-AIH overlap or SS groups; however, we cannot exclude that the patients exhibiting anti-Ro52 could have other subclinical inflammatory diseases in addition to PBC.

Most patients' quality of life was affected by fatigue, and the domain which affected the fewest was itching. The scores in our study are comparable to similar PBC cohorts.[31,32] Besides the association between the two symptoms, *dry mouth* and *dry eyes*, from the general symptom's domain, we did not find any association between PBC-40 scores and presence of anti-Ro52 or domain autoantibodies.

Limitations

The patient material is included from a tertiary referral center receiving more complicated cases, including more patients with PBC-AIH overlap syndrome, which might have influenced the results. Secondly, we use an in-house assay to detect full-length anti-Ro52 in combination with the three major domains of TRIM21. The assay use a cut-off based method for defining positivity, which could deviate from commercially available assays. We have validated our assay toward standard anti-SSA and -Ro52 assays, and our in-house assay has a specificity and sensitivity >0.9 for both. It is therefore unlikely that our cut-off impacts our results significantly.

CONCLUSION

This study found 16.7% of the PBC cohort positive for anti-Ro52, 9.7% for anti-Coiled-Coil, 5.1% for anti-PRY/SPRY, and 4.2% for anti-RING autoantibodies. We demonstrated that autoantibodies against the full-length TRIM21 protein were a significant predictive factor for SS within this cohort. The authors propose that all PBC patients should be screened for anti-Ro52 at the time of PBC diagnosis. This will help diagnose all SS patients coherently and minimize diagnostic delay, which is well-known within the SS group.

LAY SUMMARY

Primary biliary cholangitis is an autoimmune liver disease where the immune system attacks the small bile ducts inside the liver. If left untreated, it can lead to liver cirrhosis and, in some cases, the need for a liver transplant. Many patients with PBC also develop other autoimmune diseases. One of the most common is Sjögren's syndrome, which affects the moisture-producing glands and can cause dry eyes and mouth, joint pain, and severe fatigue. In this study, we tested patients with PBC to see whether they had an autoantibody commonly found in people with Sjögren's syndrome. We found that patients with PBC who had this antibody were at significantly higher risk of also having—or developing—Sjögren's syndrome. Based on these findings, we propose that all PBC patients be screened for this antibody to help identify Sjögren's syndrome earlier and improve care.

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DATA AVAILABILITY STATEMENT

All data can be made available upon reasonable requests to the authors.

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APPENDIX A

TABLE A1 PBC-40 domain (number of questions).

	None	Mild	Moderate	Severe
General symptoms (7)	<8	8–18	19–25	>26
Itch (3)	<4	4–8	9–11	>12
Fatigue (11)	<12	12–28	29–39	>40
Cognitive (6)	<7	7–15	16–21	>22
Social (10)	<11	11–28	29–40	>41
Emotional (3)	<4	4–7	8–11	>12

Note: Table A1 showing the defined domain scores for the six domains within the PBC-40 questionnaire.[20].

TABLE A2 Excluded for AIH.

		TRIM21		RING		Coiled-Coil		PRY/SPRY	
Characteristic	N	RR (95% CI)	*p*-value	RR (95% CI)	*p*-value	RR (95% CI)	*p*-value	RR (95% CI)	*p*-value
AMA positive at diagnosis	197	1.31 (0.63, 3.31)	0.5	1.63 (0.29, 30.3)	0.6	1.45 (0.51, 6.01)	0.5	0.54 (0.15, 2.48)	0.4
IgG >15.0 mg/mL	191	2.26 (1.19, 4.29)	0.014	6.03 (1.34, 41.2)	0.019	3.01 (1.25, 7.52)	0.014	7.23 (1.73, 48.4)	0.006
IgM >2.08 mg/mL	201	1.09 (0.59, 2.15)	0.8	0.95 (0.24, 4.53)	>0.9	2.28 (0.88, 7.74)	0.10	1.33 (0.38, 6.03)	0.7
Fibro scan >9.6 kPa	199	1.06 (0.46, 2.08)	0.9	0.63 (0.03, 3.37)	0.6	1.09 (0.33, 2.79)	0.9	0.49 (0.03, 2.47)	0.4
sCD163 > 3.86 mg/L	200	1.20 (0.66, 2.22)	0.5	1.60 (0.40, 7.64)	0.5	1.44 (0.62, 3.54)	0.4	1.44 (0.42, 5.49)	0.6
AIH	205	–	–	–	–	–	–	–	–
Diabetes	204	0.71 (0.12, 2.05)	0.6	–	–	–	–	–	–
Osteoporosis	204	1.11 (0.45, 2.27)	0.8	0.77 (0.04, 4.12)	0.8	1.92 (0.66, 4.64)	0.2	1.54 (0.24, 6.03)	0.6
Sjögren's syndrome	204	5.35 (2.72, 8.16)	<0.001	19.90 (2.08, 65.50)	<0.001	11.10 (5.27, 20.10)	<0.001	22.10 (7.51, 61.00)	<0.001
Myxedema	204	0.97 (0.25, 2.37)	>0.9	1.48 (0.08, 7.68)	0.7	0.57 (0.03, 2.53)	0.5	–	–
Scleroderma	204	1.17 (0.07, 3.93)	0.9	–	–	–	–	–	–
Dry eyes	193	1.81 (0.93, 3.92)	0.085	2.03 (0.48, 13.6)	0.3	1.90 (0.76, 5.69)	0.2	6.10 (1.18, 111)	0.028
Dry mouth	194	2.54 (1.19, 6.54)	0.014	1.69 (0.40, 11.3)	0.5	3.01 (1.05, 12.6)	0.04	5.08 (0.98, 92.7)	0.053
Symptoms domain	205	0.60 (0.24, 1.26)	0.2	0.43 (0.02, 2.34)	0.4	0.53 (0.13, 1.51)	0.3	0.75 (0.12, 2.90)	0.7
Fatigue domain	205	0.97 (0.52, 1.76)	>0.9	0.45 (0.07, 1.91)	0.3	0.58 (0.21, 1.39)	0.2	0.34 (0.05, 1.31)	0.12

(Continues)

TABLE A2 (Continued)

		TRIM21		RING		Coiled-Coil		PRY/SPRY	
Characteristic	N	RR (95% CI)	p-value	RR (95% CI)	p-value	RR (95% CI)	p-value	RR (95% CI)	p-value
Itch domain	205	0.39 (0.07, 1.17)	0.1	–	–	0.35 (0.02, 1.56)	0.2	–	–
Social domain	205	0.61 (0.22, 1.33)	0.1	0.54 (0.03, 2.91)	0.5	0.42 (0.07, 1.37)	0.2	0.42 (0.02, 2.14)	0.3
Emotional domain	205	0.83 (0.41, 1.54)	0.6	0.27 (0.01, 1.47)	0.15	0.63 (0.21, 1.55)	0.3	0.21 (0.01, 1.08)	0.065
Cognitive domain	205	1.16 (0.57, 2.16)	0.7	1.01 (0.15, 4.22)	>0.9	1.01 (0.34, 2.46)	>0.9	0.75 (0.12, 2.90)	0.7

Note: Table A2: Unadjusted relative risk for antibody positivity with different categorical variables as exposure for the PBC-only group without the AIH-PBC overlap group. For all PBC-40 domains, patients are divided into two groups with no symptoms (none or mild severity category) or symptoms (moderate or severe category). All risks are calculated using binomial regression.

Abbreviations: AIH: Autoimmune Hepatitis; AMA: Anti-Mitochondrial Antibodies; IgG: Immunoglobulin G; IgM: Immunoglobulin M; RR: Relative Risk.

TABLE A3 Excluded for SS.

		TRIM21		RING		Coiled-Coil		PRY/SPRY	
Characteristic	N	RR (95% CI)	p-value	RR (95% CI)	p-value	RR (95% CI)	p-value	RR (95% CI)	p-value
AMA positive at diagnosis	219	1.44 (0.64, 4.05)	0.4	1.06 (0.16, 20.5)	>0.9	1.86 (0.55, 11.6)	0.4	0.53 (0.11, 3.75)	0.5
IgG >15.0 mg/mL	212	2.37 (1.22, 4.59)	0.017	4.73 (0.95, 33.5)	0.068	3.38 (1.36, 8.94)	0.009	5.91 (1.31, 40.5)	0.031
IgM >2.08 mg/mL	224	1.15 (0.60, 2.36)	0.7	0.77 (0.17, 3.83)	0.7	2.89 (0.99, 12.2)	0.086	0.96 (0.24, 4.60)	<0.9
Fibro scan >9.6 kPa	222	1.15 (0.51, 2.24)	0.7	1.49 (0.22, 6.68)	0.6	1.43 (0.48, 3.59)	0.5	1.24 (0.19, 5.19)	0.8
sCD163 > 3.86 mg/L	224	1.07 (0.57, 2.01)	0.8	0.81 (0.16, 3.58)	0.8	1.07 (0.43, 2.65)	0.9	0.64 (0.13, 2.56)	0.5
AIH	229	0.64 (0.16, 1.65)	0.4	2.65 (0.39, 11.7)	0.2	1.33 (0.32, 3.74)	0.6	2.21 (0.33, 9.11)	0.3
Diabetes	228	0.60 (0.10, 1.81)	0.5	–	–	–	–	–	–
Osteoporosis	228	0.76 (0.24, 1.79)	0.6	–	–	1.18 (0.28, 3.40)	0.8	–	–
Sjögren's syndrome	229	–	–	–	–	–	–	–	–
Myxedema	228	0.60 (0.10, 1.81)	0.5	1.56 (0.08, 8.59)	0.7	–	–	–	–
Scleroderma	228	1.39 (0.09, 4.79)	0.7	–	–	–	–	–	–
Dry eyes	214	1.58 (0.81, 3.36)	0.2	1.89 (0.42, 13.0)	0.4	1.38 (0.55, 3.89)	0.5	5.28 (0.96, 97.8)	0.12
Dry mouth	215	2.07 (0.99, 5.01)	0.073	1.51 (0.33, 10.4)	0.6	1.96 (0.72, 6.79)	0.2	4.23 (0.77, 78.4)	0.2
Symptoms domain	229	0.48 (0.15, 1.14)	0.15	–	–	0.21 (0.01, 0.99)	0.13	–	–
Fatigue domain	229	0.97 (0.50, 1.81)	>0.9	0.23 (0.01, 1.32)	0.2	0.53 (0.18, 1.36)	0.2	0.20 (0.01, 1.09)	0.13
Itch domain	229	0.45 (0.08, 1.37)	0.3	–	–	0.42 (0.02, 1.93)	0.4	–	–
Social domain	229	0.52 (0.16, 1.23)	0.2	–	–	0.23 (0.01,1.06	0.15	–	–
Emotional domain	229	0.86 (0.41, 1.64)	0.7	–	–	0.59 (0.17, 1.57)	0.3	–	–
Cognitive domain	229	1.23 (0.59, 2.34)	0.6	1.18 (0.17, 5.31)	0.8	1.13 (0.38, 2.87)	0.8	0.98 (0.15, 4.13)	>0.9

Note: Table A3: Unadjusted relative risk for antibody positivity with different categorical variables as exposure without the patients with Sjögren's syndrome. For all PBC-40 domains, patients are divided into two groups with no symptoms (none or mild severity category) or symptoms (moderate or severe category). All risks are calculated using binomial regression.

Abbreviations: AIH: Autoimmune Hepatitis; AMA: Anti-Mitochondrial Antibodies; IgG: Immunoglobulin G; IgM: Immunoglobulin M; RR: Relative Risk.