Thyroid Dysfunction in Primary Biliary Cholangitis: A Comparative Study at Two European Centers

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OBJECTIVES: **Primary biliary cholangitis (PBC) is often associated with other autoimmune diseases, but little is known about the influence of thyroid disease (TD) on the natural history of PBC. Our aim is to analyze the association between PBC and TD, and the latter's impact on the natural history of PBC at two European centers.**

METHODS: **The study involved 921 PBC patients enrolled between 1975 and 2015 in Padova (376 patients) and Barcelona (545 patients), with a mean follow-up of 126.9±91.7 months. Data were recorded on patients' histological stage at diagnosis, biochemical data, associated extrahepatic autoimmune conditions, and clinical events, including hepatic decompensation.**

RESULTS: **A total of 150 patients (16.3%) had TD, including 94 patients (10.2%) with Hashimoto's thyroiditis; 15 (1.6%) with Graves' disease; 22 (2.4%) with multinodular goiter; 7 (0.8%) with thyroid cancer; and 12 (1.3%) with other thyroid conditions. The prevalence of different types of TD was similar in Padova and Barcelona, except for Graves' disease and thyroid cancer, which were more frequent in the Padova cohort (15.7 vs. 5.0%, and 8.6 vs. 1.3%, respectively, $P<0.05$). Overall, there were no differences between PBC patients with and without TD in terms of their histological stage at diagnosis, hepatic decompensation events, occurrence of HCC, or liver transplantation rate. The presence of associated TD was not associated with lower survival for PBC patients in either cohort.**

CONCLUSIONS: **TDs, and autoimmune TD like Hashimoto's thyroiditis in particular, are often associated with PBC, but the presence of TD does not influence the rate of hepatic complications or the natural history of PBC.**

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INTRODUCTION

Primary biliary cholangitis (formerly named primary biliary cirrhosis, PBC) is a chronic liver disease characterized by progressive immune-mediated destruction of the small-to-medium-sized bile ducts, resulting in chronic cholestasis, portal inflammation and, eventually, cirrhosis (1). It mainly affects middle-aged women, and frequently coexists with various autoimmune diseases. The probability of PBC being associated with at least one autoimmune disorder is reportedly as high as 61.1% (2), and thyroid diseases (TD) are one of the most common associations with PBC. Crowe *et al.* (3) reported positivity for thyroid antibodies in 25 of 95 patients with PBC (26%); and 13 of these patients had biochemical evidence of thyroid dysfunction, usually hypothyroidism (32%). A positive correlation emerged with thyroid antibodies and sicca syndrome, but not with histological stage of PBC. Elta *et al.* (4) studied thyroid metabolism in 58 patients with PBC and found a 22% prevalence of hypothyroidism.

The prevalence of TD in PBC reportedly ranges between 7.24 and 14.4% (2–5), the most often encountered thyroid dysfunction being Hashimoto's thyroiditis. TDs are also commonly associated with primary sclerosing cholangitis (6), and autoimmune hepatitis (7,8). The frequency of TD in autoimmune liver diseases is substantially higher than in the general population. Since fatigue, lethargy, anorexia, and hypercholesterolemia are common features

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of both hypothyroidism and PBC, patients with PBC should be screened for evidence of TD (5). In addition, little attention has been paid in the literature to the influence of TD on the natural history of PBC.

The aim of the present study conducted at two European centers was to analyze the association between PBC and TD, and the impact of the latter on the natural history of PBC.

METHODS

All PBC patients were seen prospectively and included in a database (1975–2015). A detailed description of our recruitment methods and diagnostic and follow-up criteria is available elsewhere (9). The present study concerns all consecutive PBC patients collected between 1975 and February 2015 who had a follow-up of at least 1 year. PBC was diagnosed on the basis of an antimitochondrial antibody (AMA) positivity higher than 1:40, abnormal alkaline phosphatase levels (at least 1.5 times the norm), and/or a compatible liver histology. The AMA-negative variant was diagnosed in patients with abnormal alkaline phosphatase levels (at least 1.5 times the norm), an antinuclear antibody positivity of at least 1:40, and a liver histology compatible with PBC. In AMA-negative patients, biliary tree patency was assessed by ultrasound and nuclear magnetic resonance imaging with cholangiography. Histological staging was done according to Scheuer's classification (10).

The diagnosis of Hashimoto's thyroiditis was established from a combination of clinical features, the presence of serum antibodies against thyroid antigens (mainly thyroperoxidase and thyroglobulin), and thyroid sonography (11). Graves' disease was diagnosed in the presence of one or more of the following features in a thyrotoxic patient: detectable thyroid antibodies in the serum; evidence of ophthalmopathy and/or dermopathy; and diffuse and increased thyroid radio-iodine uptake (12). Multinodular goiter was diagnosed on the basis of a clinical assessment, including physical examination; ultrasound; fine-needle aspiration biopsy to exclude malignancy; and serum TSH assay to assess thyroid function (13).

Patient follow-up and event assessment

All patients were assessed at 4- to 6-monthly intervals at the outpatients clinic by the same dedicated staff, who recorded clinical signs and symptoms of liver disease and extrahepatic autoimmunity, and laboratory parameters including aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, bilirubin, prothrombin time, alkaline phosphatase, serum albumin, IgG, IgA, IgM, total cholesterol, platelet and leukocyte counts, and hemoglobin. Alpha-fetoprotein was assessed every 6 months in patients with features of advanced disease. Liver ultrasound was performed annually in patients in the precirrhotic stage, and every 6 months in those in histological stage IV. From 1987 onwards, all patients were treated with ursodeoxycholic acid (UDCA) at a dose of 15 mg/kg/day. Only eight patients with systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) were treated with prednisolone or prednisone, tapered to a maintenance dose of 7.5 mg/day. Patients with hepatitis B or C infections were excluded for the purposes of this study. Fatigue was assessed in 339 subjects using the PBC-40 questionnaire, a fully validated, PBC-specific multidomain tool assessing quality of life (14); fatigue was scored on a 5-point scale (≤11=absent; 12–23=mild; 24–34=moderate; 35–45=severe; >45=extreme).

The mean follow-up was 126.9±91.7 months. The onset of major complications during the follow-up was classified as follows: HCC was diagnosed by ultrasound (US), computed tomography (CT), or nuclear magnetic resonance imaging (NMRI), as appropriate; ascites was identified clinically or by abdominal US; gastrointestinal bleeding due to portal hypertension was confirmed by endoscopy in cases of esophageal or gastric varices, or hypertensive gastropathy; portal-systemic encephalopathy was established from clinical parameters according to the West Haven criteria (15). The study was approved by the local ethical committee and all participants gave their informed consent to the study.

Statistical analyses

Standard descriptive statistics were used to describe the sample's characteristics. The chi-squared test was used to test for differences in categorical variables between two independent groups, as appropriate, and Student's *t*-test was used for differences in continuous variables between two independent groups. A logistic regression analysis was run (with 95% confidence intervals). Survival was analyzed with Kaplan–Meier curves (Breslow Generalized Wilcoxon test), and Cox's proportional model was applied in order to determine the presence of independent risk factors that may influence survival besides the presence of thyroid dysfunctions. A P value ≤0.05 was considered statistically significant. The statistical analysis was conducted using SPSS software (Chicago, IL, USA).

RESULTS

Table 1 shows the clinical characteristics of the study group. The two series of patients had similar demographic and clinical characteristics, except for smoking habit, with significantly more smokers in Padova than in Barcelona (19.9 vs. 12.9%, $P<0.05$). There were more patients in histological stage III in Padova than in Barcelona (25.8 vs. 10.6%, $P<0.05$). There was no difference between the two cohorts regarding the prevalence of decompensation events during the follow-up (ascites, variceal bleeding, spontaneous bacterial peritonitis, hepatic encephalopathy, and hepatocellular carcinoma).

Among the 921 PBC patients, 150 (16.3%) had an associated TD, 117/921 (12.7%) with and 33/921 (3.6%) without an autoimmune etiology. No significant differences emerged between the two cohorts regarding the presence of TD. Overall, 94 patients (10.2%) had Hashimoto's thyroiditis, 15 (1.6%) had Graves' disease, 22 (2.4%) had multinodular goiters, 7 (0.8%) had thyroid cancer, and 12 (1.3%) had other forms of TD. Autoimmune TD was identified in 117 PBC patients (78% of the 150 patients with associated TD), with a similar prevalence in the two cohorts (78.6 and 77.5% in Padova and Barcelona, respectively). **Table 2** shows the different types of TD in the study population and for the two

Table 1. Clinical characteristics of the study group

Characteristic	Padova	Barcelona	Total	*P*
M/F	25/351	42/503	67/854	n.s.
Mean age (years)	68±14	70±16	69±15	n.s
Mean age at diagnosis (years)	53.2±12.3	51.2±13.1	52.2±12.8	n.s
Mean FU (months)	115.2±85.6	135.0±94.9	126.9±91.7	n.s.
AMA+	307 (81.7%)	387 (87.6%)	694 (84.9%)	n.s
UDCA	352 (93.6%)	409 (92.5%)	761 (93%)	n.s.
Smoking	75 (19.9%)	57 (12.9%)	132 (16.1%)	<0.05
Alcohol >40 g/die	12 (3.08%)	19 (3.3%)	31 (3.3%)	n.s.
Histological stage at diagnosis				
I	113 (30.1%)	251 (46.1%)	364 (39.5%)	n.s.
II	70 (18.6%)	110 (20.2%)	180 (19,5%)	n.s.
III	97 (25.8%)	58 (10.6%)	155 (16.8%)	<0.05
IV	29 (7.7%)	40 (7.3%)	69 (7.5%)	n.s.
Biopsy not performed	67 (17.8%)	86 (15.8%)	153 (16.6%)	n.s.
Biochemical data at diagnosis				
AST (×ULN)	1.67±1.21	1.75±1.3	1.71±1.26	n.s.
ALT (×ULN)	1.87±1.45	2.18±1.79	2.05±1.67	n.s.
GGT (×ULN)	5.85±5.56	6.47±6.32	6.22±6.02	n.s.
ALP (×ULN)	2.47±2.3	2.51±2.17	2.49±2.22	n.s.
Bilirubin (mg/dl)	0.74±0.73	1.39±2.68	1.13±2.14	n.s.
PTL (×10^9/l)	241.94±85	238.88±92.02	240.1±89.23	n.s
Albumin (mg/dl)	41.42±5.91	41.6±4.85	41.52±5.31	n.s
IgG (mg/dl)	1524±577	1602±610	1597±608.23	n.s.
IgA (mg/dl)	306.63±130.3	315.6±237.73	315.13±233.3	n.s.
IgM (mg/dl)	353±179.89	498.52±591.66	489.16±575.1	n.s.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, antimitochondrial antibodies; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; Ig, immunoglobulins; n.s., not significant; PT, prothrombin time; PTL, platelets; UDCA, ursodeoxycholic acid; ULN, upper normal limit. Values are expressed as mean±standard deviation of the total.

Table 2. Types of thyroid disease (TD) in 150 patients from Padova and Barcelona

Thyroid disease type	Padova	Barcelona	Total	*P*
Hashimoto's thyroiditis	42 (60.0%)	52 (65.0%)	94 (62.7%)	n.s.
Graves' disease	11 (15.7%)	4 (5.0%)	15 (10.0%)	*P*=0.03
Multinodular goiter	10 (14.3%)	12 (15.0%)	22 (14.7%)	n.s.
Thyroid cancer	6 8.6%)	1 (1.3%)	7 (4.7%)	*P*=0.03
Others	3 (4.3%)	9 (11.5%)	12 (8.0%)	n.s.

n.s., not significant.

cohorts. The prevalence of the different types of TD was similar in Padova and Barcelona, except for Graves' disease and thyroid cancer, which were both more common in the Padova cohort (15.7 vs. 5.0%, and 8.6 vs. 1.3%, respectively; $P<0.05$). The difference in the thyroid cancer rate between the two cohorts is further discussed below. Among the 150 PBC patients with TD, 34 (22.6%) had been diagnosed with TD before their PBC was diagnosed, whereas 116 (77.4%) developed TD during their follow-up for PBC.

No differences emerged in the biochemical data between patients with and without TD at the time of their PBC being diagnosed (**Table 3**). Fatigue was recorded in 86 patients with associated TD (57.3%), and in 410 patients without TD (53.2%); the difference was not statistically significant. A sub-analysis on fatigue was performed in 339 patients in the Barcelona cohort, using the PBC-40, a self-administered questionnaire validated for PBC. No differences emerged between patients with and without TD in terms of the association with fatigue (either as a symptom *per se*, or classified by severity (data not shown). No differences were found between PBC patients with or without TD regarding liver decom-

pensation events or the onset of HCC during the follow-up. Liver transplantation was performed in more patients in Barcelona than in Padova (9.7 vs. 1.9%, $P<0.001$), but the presence of TD did not significantly influence the indication for liver transplantation. TDs were associated with AMA-negative significantly more than with AMA-positive PBC patients (22.8 vs. 15.3%, $P=0.04$). As expected, females had a higher frequency of TD than males (17.1 vs. 5.1%, $P=0.001$). No significant differences in the rate of association with TD were found for different histological stages of PBC on presentation. Elderly age (>65 years) was also unassociated with a greater frequency of TD in our sample.

In both cohorts, in Padova and Barcelona, response to UDCA was assessed after 1 year of therapy according to the following criteria: Barcelona (16), Paris I (17), Paris II (18), and Rotterdam (19). The rate of responders ranged between 67 and 83% (**Table 4**). No significant difference in the response rate was found between patients with and without associated TD (**Table 4**).

In our study population, 147 patients died during the follow-up, 66 (21.3%) in Padova and 81 (18.2%) in Barcelona (P=n.s.). The mean global transplant-free survival rate was 314.4±9.2 months. The mean transplant-free survival rate of PBC patients with and without associated TD was 345.4±21.6 months, and 305.8±10.1 months, respectively; the difference was not significant (**Figure 1**). Using Cox's regression analysis, adjusted for sex and age, survival was not influenced by any associated TD or another extrahepatic autoimmune condition, SLE or scleroderma/CREST or RA, smoking habit or alcohol consumption (>40 g/day), or histological stage at diagnosis (**Table 5**). Survival was also similar in patients with and without TD, irrespective of their response to UDCA (P=n.s.)

The overall survival rate was similar in the two cohorts in Padova and Barcelona, but when patients without TD were considered separately, those in the Padova cohort had a significantly lower survival rate than those in Barcelona (248.2±14.3 vs. 320.3±9.3 months, $P=0.001$).

Table 3. Biochemical data at diagnosis according to the presence of thyroid disease (TD)

Variable	Patients with TD (N. 150)	Patients without TD (N. 771)	*P*
AST (×ULN)	1.71±1.2	1.67±1.24	n.s.
ALT (×ULN)	2.1±1.69	1.99±1.6	n.s.
GGT (×ULN)	6.04±6.07	5.98±5.8	n.s.
ALP (×ULN)	2.32±2.15	2.43±2.22	n.s.
Bilirubin (mg/dl)	1.08±2.51	1±1.89	n.s.
Albumin (mg/dl)	41.7±4.92	41.6±5.22	n.s.
PTL (×10^9/l)	246±86.11	237±90.11	n.s.
IgG (mg/dl)	1629±606.13	1539±581.23	n.s.
IgA (mg/dl)	284±155.56	304±255.37	n.s.
IgM (mg/dl)	465±306.48	447±635.67	n.s.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; Ig, immunoglobulins; n.s., not significant; PTL, platelets; ULN, upper normal limit. Values are expressed as mean±standard deviation of the total.

DISCUSSION

The results of our study confirm that TD are often associated with PBC, especially Hashimoto's thyroiditis, which shares an autoimmune etiology with PBC. There were no substantial differences in the prevalence–incidence of TD among PBC patients at the two European centers investigated. More importantly, the presence of TD in a PBC patient did not influence the rate of hepatic complications or response to UDCA treatment. Indeed, the clinical

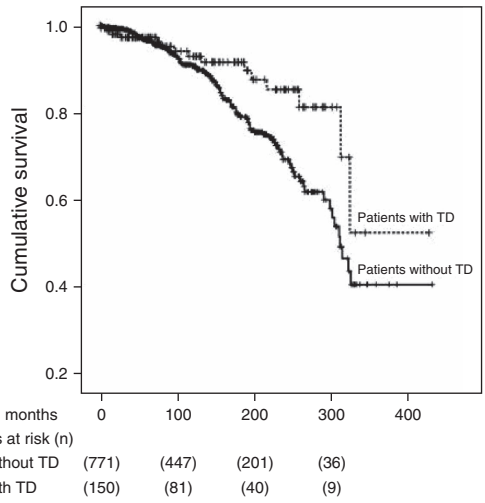

Figure 1. Kaplan–Meier curves for transplant-free survival in patients with and without TD (Breslow generalized Wilcoxon test).

Table 4. Response to UDCA treatment according to the International criteria in patients with and without TD

Criteria	UDCA responders with TD	UDCA responders without TD	*P* value	OR (95% CI)
Barcelona	100 (67%)	497 (64%)	n.s.	1.1162 (0.7198–1.7308)
Paris I	105 (69%)	568 (74%)	n.s.	0.8258 (0.5423–1.2576)
Paris II	124 (83%)	525 (69%)	n.s.	0.8471 (0.5667–1.2664)
Rotterdam	121 (81%)	578 (75%)	n.s.	0.6088 (0.3425–1.0823)

n.s., not significant.

Table 5. Multivariate analysis for survival adjusted for sex and age

	P value	Exp (B)	95% CI for exp (B)
Smoking	0.498	1.229	0.677–2.230
Alcohol consumption >40 g/day	0.615	0.849	0.449–1.607
Histological stage at diagnosis	0.606	1.046	0.883–1.238
Extrahepatic autoimmunity	0.840	1.043	0.694–1.568
Systemic lupus erithematosus	0.858	1.140	0.271–4.798
Scleroderma/CREST	0.975	1.011	0.494–2.072
Rheumatoid arthritis	0.379	0.684	0.294–1.594

Exp=hazard ratio.

characteristics and natural history of PBC were much the same in the two cohorts, as demonstrated by the absence of significant differences regarding histological stage at diagnosis (the only exception being more patients in stage III in the Italian cohort); biochemical data; response to UDCA; the association with other extrahepatic autoimmune disorders; the occurrence of clinical events; and survival. The cohort from Padova included fewer patients who received a liver transplant, and there are several reasons for this difference, including the age cutoff for transplantation (which was strictly 60 years up until 2000), and the number of patients dying on the waiting list.

The prevalence of TD is estimated to be higher in the PBC population than in the general population both in Italy and in Spain, though no specific epidemiological data on autoimmune and non-autoimmune TDs have been published for these two European countries to date. As expected, Hashimoto's thyroiditis (HT) was the most common TD in both cohorts (11.2% in Padova and 9.5% in Barcelona). Two small studies reported a 3.5% prevalence of HT in Spain (20), and 5% in Italy (21). It has been estimated that up to 5% of the general population have autoimmune TD (22,23). In the Whickham study, conducted in Northern England, the prevalence of spontaneous hypothyroidism from Hashimoto's thyroiditis was 15/1,000 in women (who were diagnosed at a mean 57 years of age), and less than 1/1,000 in men (22). In a 3-year survey of 1,457,036 inhabitants of a city in Sweden, the incidence of Graves' disease (GD) was 24.5/100,000 a year, and the condition peaked among those aged 30–39 years (24). To date, no published studies have reported on the prevalence of non-autoimmune thyroid conditions in PBC.

The HT rate in our sample was similar to the prevalence previously reported in other PBC series from different geographical areas (7,25,26), and in other autoimmune disorders as well. The high prevalence of HT in autoimmune diseases, including PBC, may reflect a common pathogenic mechanism targeting different organs. In fact, the probability of a patient with PBC having another autoimmune disorders is as high as 61.1% (2). On the other hand, multinodular goiter showed an overall prevalence of 2.4% in the PBC population, with no significant difference between Padova and Barcelona. The rate of multinodular goiter in the general population varies considerably by geographical area, depending mainly on the population's iodine intake. Both Padova and Barcelona are mildly iodine-deficient areas. The Whickham study found a 5.9% prevalence of multinodular goiter in the UK (22).

We found a significant difference in the prevalence of GD between the two centers considered (0.7% in Barcelona, 2.9% in Padova; $P<0.05$). These figures are much the same as the prevalence of GD in the general population in Spain, and slightly higher than the 1.85% for the population at large in Italy (21). The prevalence of thyroid cancer was also higher in the PBC cohort than in the general population in Padova, confirming a previously reported observation (27), whereas the prevalence of thyroid cancer in the Barcelona cohort was negligible (0.2%). It may be that the higher prevalence of thyroid cancer in the Italian cohort reflects the geographical distribution of this neoplasm: Italy has one of the highest incidences of thyroid cancer (19.6 per 100,000 person years in females), whereas the incidence of this cancer in Spain is more than halved (7.8 per 100,000 person years in females) (28). These figures, drawn from the two countries' national cancer registries, suggest that thyroid cancer might have no correlation with PBC.

Our study showed that an associated TD had no influence on the natural history of PBC or on patient survival. When our two cohorts were considered separately, the presence of TD correlated with a better survival for PBC patients in Padova, but not in Barcelona, but the limited information available on the causes of death prevented us from investigating this issue adequately. It should be noticed that treatment with levothyroxine (used to correct clinical hypothyroidism) significantly affects a patient's inflammatory profile, reducing the pro-inflammatory cytokines, increasing IL-10 levels, and lowering serum IgG concentrations (29,30). Levothyroxine therapy might therefore improve coronary microvascular function and thereby prevent cardiovascular events (31–33).

The present study has some obvious limitations. In particular, the study design suffers from some methodological bias: the database was collected on the grounds of the same criteria, and all medical histories were checked by consulting patients' medical records concerning thyroid dysfunction, but this procedure was not standardized. In addition, the lack of data on causes of death should be considered a bias in our survival analysis.

In conclusion, TDs—and particularly HT—are often associated with PBC, but do not influence the rate of hepatic complications or the natural history of the latter disease.

CONFLICT OF INTEREST

Guarantor of the article: Annarosa Floreani, MD.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Primary biliary cholangitis (PBC) is an autoimmune liver disease with a 60% probability of being associated with other extrahepatic autoimmune conditions.
- ✓ Thyroid diseases are often associated with autoimmune conditions in women.

WHAT IS NEW HERE

- ✓ Associated thyroid dysfunctions are found in 16.3% of patients with PBC. Besides Hashimoto's thyroiditis, clinicians should check for Graves' disease, non-autoimmune thyroid diseases and thyroid cancer.
- ✓ Any associated thyroid diseases do not influence the rates of hepatic complications or transplant-free survival in PBC patients.

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