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Extrahepatic Malignancies in Primary Biliary Cirrhosis: A Comparative Study at Two European Centers

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Abstract Limited information and divergent results are available on the prevalence/incidence, survival, and risk factors for developing extrahepatic malignancies (EMs) in primary biliary cirrhosis (PBC). The aim of the study was to analyze the epidemiology and survival rates for EM in PBC patients. The study was conducted on two series of patients followed up at two European centers (361 in Padova, Italy, and 397 in Barcelona, Spain) for a mean 7.7±7 and 12.2±7 years, respectively. The cancer incidence was compared with the standardized incidence ratios (SIRs) calculated using the Cancer Registry of the Veneto Region (Italy) and the Cancer Registry of Tarragona (Spain). Seventy-two patients developed EM. The prevalence of cases was similar in Padova (9.7 %) and Barcelona (9.4 %). The overall cancer incidence was similar to the expected incidence for the general population in the same geographical area (SIR=1.2), and so was the crude EM rate (855.01 vs 652.86 per 100,000 patient-years, respectively, RR=1.3). Logistic regression analysis showed that advanced histological stage and extrahepatic autoimmune diseases were significantly associated with the onset of EM. Survival was similar for PBC patients with and without EM (p=n.s.), and actual survival was similar to the one predicted by the Mayo model. The incidence of EM in PBC patients was found similar in Italy and Spain and no different from that of the general population. Advanced histological stage and extrahepatic autoimmune disease were risk factors significantly associated with EM developing in PBC. The onset of cancer in PBC patients does not influence the natural history of their liver disease.

Keywords Extrahepatic malignancies · PBC · Cancer · Survival

Abbreviations

EM	Extrahepatic malignancies
PBC	Primary biliary cirrhosis
AMA	Antimitochondrial antibodies
HCC	Hepatocellular carcinoma
UDCA	Ursodeoxycholic acid
SIRs	Standardized incidence ratios
IARC	International agency for research on cancer

Introduction

The association of cancer with autoimmune disease is still debated. For the past three decades, reports have suggested an increased cancer risk in many autoimmune disease; the most important studies regard rheumatoid arthritis (RA), Sjogren's syndrome (SS), and systemic lupus erythematosus (SLE) [1, 2]. As far as autoimmune liver disease is concerned, it is well known that liver cancer may develop during the follow-up as a

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complication of cirrhosis, analogously to different liver disease from different etiologies.

Nevertheless, the incidence of extrahepatic cancer has been studied only partially. A recent study that investigated the risk of malignancy on population-based cohorts of autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC) in Canterbury, New Zealand, found that the risk of hepatic and extrahepatic malignancy was significantly increased in AIH and PSC patients [3]. The risk of hepatocellular carcinoma (HCC) among patients with AIH and cirrhosis has been found to be comparable to HCC risk among patients with cirrhosis secondary to HBV, HCV, hemochromatosis, or alcohol-related liver disease in a large tertiary-care community hospital in Japan [4]. The risk of extrahepatic cancer has hitherto been examined in only one study in patients with AIH [5]. Four hundred and seventy-three patients with AIH were matched to the Swedish national cancer register as well as to the death cause register. An overall increased risk of malignancy, mainly caused by hepatobiliary cancers, was found; moreover, a significantly higher risk of lymphomas was found [5].

The risk of hepatic and extrahepatic malignancies has been assessed in a cohort study comprising 604 patients with PSC identified between 1970 and 1998 in Sweden [6]. In this study, the risk of hepatobiliary malignancy was increased 161 times, for colorectal carcinoma 10 times and for pancreatic carcinoma 14 times, compared with that of the general population. More recently, in a large multi-institutional cohort of patients with PSC and inflammatory bowel disease from the Massachussetts State, USA, it has been demonstrated that PSC is associated with an increased risk of mortality and cancer [7]. In this study, the authors confirmed the previously reported association with colorectal, hepatobiliary, and pancreatic cancer but failed to identify any additional association with extraintestinal tumors in patients with PSC.

PBC is a chronic liver disease with a predilection for the female gender, characterized by destruction of the bile ducts that eventually leads to cirrhosis [8]. The etiology of PBC is unknown, although several key observations have greatly advanced our understanding of the pathogenic mechanisms behind it. While genome-wide association studies have revealed the importance of genetic background [9, 10], the role of immunological response targeting the destruction of biliary epithelial cells can explain the loss of tolerance that leads to autoimmune damage [11]. Several studies have suggested that PBC may be associated with a greater risk of HCC, especially in end-stage liver disease [12–14]. Other risk factors possibly related to HCC include male gender and lack of response to ursodeoxycholic acid (UDCA) [15]. As for extrahepatic cancers, the association between PBC and malignancy is still debated. A recent meta-analysis on PBC and cancer risk, which considered approximately 16,300 PBC patients from several countries, found PBC closely associated with a higher overall risk of malignancy, and this applied particularly to HCC, but not to other extrahepatic cancers [16]. A significant association between PBC and breast cancer was reported in North America in studies conducted before the 1990s [17–19], but this association was not confirmed in more recent reports, including our own experience [20–22]. Data on extrahepatic cancer and PBC are generally difficult to interpret. It is intriguing, for example, that the incidence of thyroid tumors in PBC is near nil, even in large series of female PBC patients. There are also numerous biases that may explain why such different results are found in different countries, including a different epidemiology of risk factors for cancer, genomic bias, a different duration of the natural history of PBC, the role of treatments (UDCA has a chemoprotective effect against some cancers), and immunosuppressive agents used before 1990, as suggested by Piscaglia and Sangrini [23, 24]. In addition, and in both cancer and autoimmunity, there are not only genetic but also epigenetic and microflora influences [25–28]. The present study contributes to the body of information on the prevalence, incidence, and risk factors for extrahepatic malignancies (EMs) in PBC patients followed up at two European centers (Padova in Italy and Barcelona in Spain).

Methods

The study was conducted on two series of patients with PBC at two European centers (the Gastroenterology Section of the Department of Surgery, Oncology and Gastroenterology at the University of Padova in Italy and the Liver Unit at the Digestive Disease Institute, Hospital Clinic, IDIBAPS, University of Barcelona in Spain), comprising a total of 753 patients (361 in Padova and 392 in Barcelona) who were followed up for a mean of 10.4±7.3 years (7.7±7 and 12.2±7 years in Padova and Barcelona, respectively). Data on the total patient population at diagnosis are shown in Table 1. The two series were similar in terms of age and gender, but patients in Barcelona had higher bilirubin levels (2.4±4.1 vs 0.7±0.9 for the patients in Padova, $p<0.05$) and Mayo score (4.5±1.1 vs 3.8±1.0, $p<0.05$), while the Italian series included more patients with stage III disease at diagnosis and more smokers (respectively, 32 vs 14 %, $p<0.05$, and 20 vs 7 %, $p<0.05$). The majority of patients (87 %) were treated with UDCA at a mean daily dosage 15 mg/kg of body weight.

PBC was diagnosed if patients met two of the following criteria: alkaline phosphatase at least 1.5 times upper normal levels, positive antimitochondrial antibodies (AMA), and a compatible liver biopsy. Patients were assessed regularly every 4–6 months, using clinical and analytical procedures. A detailed medical history was recorded when PBC was

Table 1 Demographic, clinical, biochemical, immunological, and histological characteristics of patients when their primary biliary cirrhosis (PBC) was diagnosed

Characteristic	*N*=753
Age (years)	52.4±12.7
Female (%)	93
AMA-positive (%)	85
Total bilirubin (mg/dL)	1.6±3.7
ALT (xULN)	2.2±1.6
γ-GT (x ULN)	6.8±6.6
Alkaline phosphatase (x ULN)	2.7±2.4
Albumin (g/L)	4.1±0.6
Prothrombin time (s)	12±1.6
Platelet count (x 10^9/L)	226±83
Histological stage (%)	
I	46
II	23
III	22
IV	9
Mayo risk score	4.2±1.1
HCV prevalence (%)	3
Smokers (%)	12
Alcohol intake >40 gr/day (%)	1.4

Quantitative variables are expressed as mean±standard deviation

AMA antimitochondrial antibody, *ALT* alanine aminotransferase, *γ-GT* gamma-glutamyl transferase, *ULN* upper limit of normal, *HCV* hepatitis C virus

diagnosed, including any history of previous malignancies, and this information was updated at each visit.

The following features were recorded as potential risk factors for the onset of EM: tobacco consumption, alcohol intake higher than 40 g/day, advanced histological stage (stages III and IV), age, antinuclear antibodies (ANAs), and extrahepatic autoimmune disease. For the Italian cohort, body mass index and family history of neoplasia were also recorded. Abdominal ultrasound was performed for all patients diagnosed after 1981 to disclose any development of HCC; it was repeated every 6 months in patients diagnosed before 1999 and at intervals ranging from 6 to 24 months (depending on histological stage) in patients assessed afterward. Histological stage was classified according to the Ludwig and Scheuer criteria in Spain and Italy, respectively [29, 30].

Statistical Analysis

Data were expressed as means±standard deviations of the mean. The chi-squared test was used to analyze differences in discontinuous variables, and Student's *t* test was used to compare continuous variables.

The standardized incidence ratio (SIR) was the ratio between observed and expected cases of cancer, and the 95 % confidence interval was based on Poisson distribution. The SIRs for tumors were calculated taking for reference: (i) the Cancer Registry of the Veneto Region, Italy, from 1987 to 2006 and (ii) the Cancer Registry of Tarragona, Spain, from 1980 to 2001. The person-year follow-up was calculated using STATA software, version 10 (StataCorp LP, College Station, TX, USA), using the following input data: (i) the date when PBC was diagnosed if it was subsequent to the period of cancer registry coverage or (ii) the date of starting the cancer registry coverage if it was subsequent to the date of diagnosis. The exit date was the date when the cancer registry coverage ended or the date when cancer was diagnosed, or the patient died, or was lost to follow-up, whichever came first.

In the SIR analysis, the general population covered by the two tumor registries was taken as the reference, and the expected number of cases were calculated from the specific incidence rates of each kind of tumor in the region on the basis of the person-years, gender, age (15–19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, 75–79, 80–84, and 85+years) and the calendar period (1980–1983, 1983–1986, 1987–1991, 1992–1997, 1997–2001, and 2002–2006).

The independent effect of significant variables associated with the development of EM was ascertained using the stepwise logistic regression test and assessing the following variables: age at diagnosis of PBC, tobacco consumption, alcohol intake higher than 40 g/day, advanced histological stage (stages III and IV), ANA, and extrahepatic autoimmune diseases. Survival without liver transplantation was analyzed from the diagnosis of PBC until death or liver transplantation. Survival curves were compared between patients who did or did not develop EM. Survival after an EM was diagnosed was calculated too and compared with the survival predicted by the Mayo model. Survival related to follow-up time was analyzed using the Kaplan-Meier method and compared using the log-rank test. A value of $p \leq 0.05$ was considered statistically significant.

Results

Ninety-nine patients (13.1 %) had a diagnosis of cancer, 44 (12.2 %) in Padova and 55 (14 %) in Barcelona. Twenty-nine of these patients (3.9 %) were identified as having HCC and were excluded from any further analyses. In all, 72 patients (9.6 %) developed EM (61 females, 3 males; 35 from Padova and 37 from Barcelona), 2 of them developing both HCC and EM. Among these 72 patients with EM, 70 had one extrahepatic tumor diagnosed, 1 had two, and 1 had three, for a total of 75 EMs.

Table 2 Tumors of reproductive organs developing in 701 female PBC patients

	Total (n=701)		Italy (n=339)		Spain (n=362)		p Value
	N°	%	N°	%	N°	%	
Breast	24	3.4 %	9	2.6 %	15	4.1 %	n.s.
Endometrium	4	0.5 %	2	0.6 %	2	0.5 %	n.s
Cervix	2	0.3 %	2	0.6 %	0	0.0 %	n.s.
Ovary	1	0.1 %	0	0.0 %	1	0.3 %	n.s.
Total	31	44.2 %	13	38.3 %	18	49.7 %	n.s.

EM was diagnosed before PBC in 21 cases (28 %), in the same year as the PBC in 6 (8 %) and after the liver disease had been diagnosed in 48 (64 %). The mean age at the time of the EM diagnosis was 60±14 years, and it was significantly higher in the Spanish than that in the Italian cohort (63±12 vs 57±12 years, p<0.05). In all, there were 31 EM involving the reproductive organs (out of a total number of 701 female PBC patients) and 44 at various other sites (Tables 2 and 3).

The prevalence of cancer was similar in Padova (9.7 %) and Barcelona (9.4 %) and so was the crude rate of EM (855.01 vs 652.86 per 100,000 patient-years, respectively; RR=1.3 [95 % confidence interval (CI) 0.75–2.21]). The overall incidence of EM in the study population was similar to the incidence expected in the general population in the same geographical area (SIRs=1.2 [95 % CI 0.9–1.5]), though a significantly higher SIR was observed in PBC patients for Hodgkin's disease and kidney cancer (Table 4). The independent effect of the clinical variables, adjusted for covariate lifestyle variables, age at diagnosis of PBC, smoking, alcohol consumption >40 g of ethanol/day, histological stage, body mass index, extrahepatic autoimmune diseases, family history of cancer, associated with the development of EM, was assessed using a logistic regression. Logistic regression analysis showed that advanced histological stage (stage IV, odds ratio 11.07 [95 % CI 2.86–42.93], p<0.001) and associated extrahepatic autoimmunity (odds ratio 2.73 [95 % CI 0.99–7.48], p<0.05) were risk factors independently associated with the development of EM (Table 5). Survival after PBC was diagnosed was much the same for cases with and without EM (29.2 and 33.4 years, respectively, p=n.s.) (Fig. 1), and PBC patients' actual survival was similar to the one predicted with the Mayo model.

Discussion

The results of this study indicate that EM can occur in PBC with much the same incidence in Italy and Spain, and that advanced histological stage (stage IV) and extrahepatic autoimmunity are the main factors independently associated with the onset of cancer. The overall cancer incidence was found similar in PBC patients to that of the general population, with the exception of Hodgkin's lymphoma and kidney cancer, which were found more frequently in PBC patients than in the general population. The occurrence of EM also had no influence on the natural history of the liver disease.

Previous reports on cancer associated with PBC were limited as regards prevalence/incidence, and the studies were

Table 3 EM (except for reproductive tumors) developing in 753 PBC patients in Padova and Barcelona

	Total (n=753)		Italy (n=361)		Spain (n=392)		p Value
	N°	%	N°	%	N°	%	
Lymphoproliferative tumors	12	1.6 %	5	1.4 %	7	1.8 %	n.s.
Colorectal cancer	10	1.3 %	3	0.8 %	7	1.8 %	n.s.
Kidney cancer	5	0.7 %	3	0.8 %	2	0.5 %	n.s.
Thyroid cancer	5	0.7 %	5	1.4 %	0	0.0 %	<0.05
Melanoma	3	0.4 %	3	0.8 %	0	0.0 %	n.s.
Skin (squamous cell carcinoma)	2	0.3 %	2	0.6 %	0	0.0 %	n.s.
Lung cancer	2	0.3 %	1	0.3 %	1	0.3 %	n.s.
Sarcoma	2	0.3 %	0	0.0 %	2	0.5 %	n.s.
Cholangiocarcinoma	1	0.1 %	1	0.3 %	0	0.0 %	n.s.
Stomach (adenocarcinoma)	1	0.1 %	0	0.0 %	1	0.3 %	n.s.
Pancreatic cancer	1	0.1 %	1	0.3 %	0	0.0 %	n.s.
Total	44	5.8 %	24	6.6 %	20	5.5 %	n.s.

Table 4 Estimated SIRs and lower/upper bounds of 95 % confidence intervals

Malignancy	Observed	Estimated	SIR	95 % CI
All cancers	62	53.6	1.2	0.9–1.5
Hodgkin's disease	2	0.1	15.5	3.9–61.9
Myeloma	2	0.7	2.8	0.7–11.0
Non-Hodgkin's lymphoma	3	1.7	1.8	0.6–5.5
Colon cancer	6	4,4	1.4	0.6–3.0
Kidney cancer	3	1.0	3.0	1.0–9.4
Breast cancer	10	14.2	0.7	0.4–1.3
Thyroid cancer	2	0.8	2.4	0.6–9.5

heterogeneous and performed mostly before the 1990s. In a recent meta-analysis pooling 17 studies [16], Liang et al. found PBC closely associated with a greater overall risk of cancer, but this mainly concerned HCC, not other cancers; the authors concluded that the association between PBC and the risk of various cancers needs to be further confirmed in future studies.

The long-term follow-up obtained at two referral centers for PBC in Italy and Spain for a very large series of PBC patients assessed over a very lengthy period of time enables us to extend the body of knowledge on the prevalence/incidence of EM and the related risk factors in patients with PBC. The two series were similar in terms of size, recruitment, follow-up, and severity of liver disease, and they both enabled to previously analyze the prevalence/incidence of HCC [12].

The prevalence of EM was similar in Padova (9.7 %) and Barcelona (9.4 %) and so was the crude cancer rate, with 855.01 versus 652.86 per 100,000 patient-years in Italy and Spain, respectively. The relatively higher incidence recorded in Italy was not statistically significant and can be explained by a shorter mean patient follow-up (7.7±7 vs 12.2±7 years in Barcelona). On pooling together the two series, we found an incidence of 728.3 per 100,000 person-years, which is slightly lower than that in other European or North American studies [20–22]. The overall incidence of EM in the study population was similar to the expected incidence of EM in the general population in the same geographical area, with two exceptions concerning Hodgkin's lymphoma and kidney cancer.

There are no reports in the literature to support any potential association between PBC and [31] lymphoma; only sporadic cases of cutaneous T cell lymphoma or MALT lymphoma have been described in PBC patients [18, 21]. In an extensive follow-up of 2,912 patients assessed at the Mayo Clinic, evidence of lymphoma was only found in 0.6 % of patients [31]. No specific association has been reported in the literature for kidney cancer either. Our results need to be confirmed by an extensive follow-up of large series of PBC patients in Europe and the USA, however, where studies are already ongoing. It should also be speculated that SS which is frequently associated with PBC might add a risk for the incidence of lymphoproliferative disorders in PBC [32]. Interestingly, a retrospective analysis of 1,320 patients with SS recruited at Peking Union Medical College Hospital from 1990 to 2005 and followed up for a mean of 4.4 years revealed that 2.2 % developed neoplasms [33]. Among these, SIR for lymphoma was 48.1, and the relative risk analysis showed that lymphoadenopathy, enlargement of parotid glands, monoclonal immunoglobulins, and absence of hypergammaglobulinemia were significantly correlated with malignancies [33].

As far as breast cancer is concerned, we confirmed previously presented findings that failed to identify any particular

Table 5 Logistic regression analysis

		Odds ratio	*p* Value	95 % CI
Histological stage at diagnosis	1	1.00		
	2	1.10	0.14	0.28–4.26
	3	2.28	0.15	0.74–7.02
	4	11.07	0.001	2.86–42.93
BMI		1.22	0.55	0.64–2.3
Age at diagnosis		0.99	0.71	0.95–1.03
Smoking	Yes	1.69	0.31	0.61–4.65
Alcohol	Yes	1.67	0.34	0.59–4.75
Extrahepatic autoimmunity	Yes	2.73	0.05	0.99–7.48
History of familiar cancer	Yes	2.25	0.08	0.91–5.56

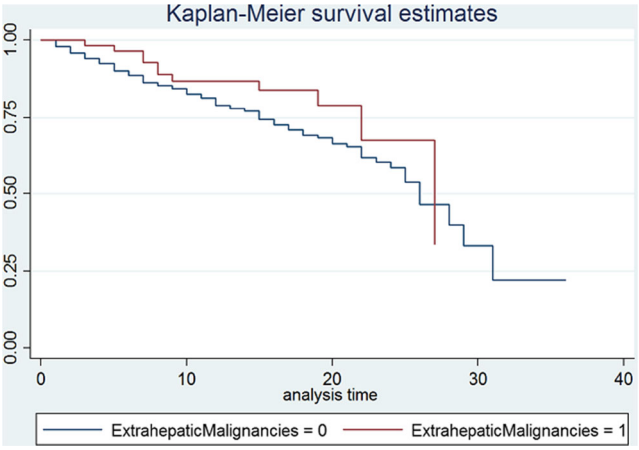

Fig. 1 Survival probability after PBC has been diagnosed in patients with and without EM

association between PBC and breast cancer [20–22]. There may be several reasons for these conflicting results vis-à-vis earlier publications on this issue. For a start, the first reports showing a higher incidence of breast cancer in PBC patients were published before the 1990s, i.e., before any screening programs for breast cancer had been introduced in Italy and Spain. The estimated cancer mortality rate has since dropped in both countries by 36 % [34], and women diagnosed more recently with breast cancer are more likely to be cured than those diagnosed in the past [35].

Interestingly, we did not observe any significant reduction in incidence of colorectal cancer in our study group. Conversely, the incidence of colorectal cancer in our study is higher than the estimated incidence reported by IARC (http://www.iarc.fr; observed/expected incidence=1.6, 95 % CI 1.3–1.8). This may be due to the extensive screening programs implemented in Europe for colorectal cancer since 1990. Like breast cancer screening, these programs enable the secondary prevention of colorectal cancer and consequently coincide with a rising trend in the diagnosis of this tumor. Another consideration concerns any chemopreventive effect of UDCA therapy on this tumor. Pardi et al. reported a significantly lower risk of colorectal dysplasia or cancer developing in 52 patients with ulcerative colitis and PSC [36]. A subsequent observational study conducted in France on 114 PBC patients enrolled in a colonoscopic surveillance program demonstrated that the prolonged administration of UDCA was unassociated with any increased prevalence of colorectal adenomas and that it significantly reduced the probability of colorectal adenoma recurrence following polypectomy [37]. It has been suggested that a protective effect could stem from a significantly reduced colon epithelial cell proliferation in patients treated with UDCA [37]. The topic is intriguing, however, because the long-term use of high-dose UDCA seems to be associated with an increased risk of colorectal neoplasia in patients with ulcerative colitis and PSC [38, 39]. No studies have been published on the long-term follow-up of PBC patients screened specifically for colorectal cancer, but our study fails to support any protective action of UDCA on the development of colorectal cancer in PBC patients.

The significant risk factors for the onset of cancer emerging from our logistic regression analysis were advanced histological stage and extrahepatic autoimmunity. It has been well documented, however, that the cancer risk is higher in patients with various autoimmune rheumatic diseases [40, 41]. This is an important issue for the management of PBC, and the autoimmune involvement of extrahepatic organs should be carefully assessed in all PBC patients [42, 43]. The most clearly documented association between cancer and rheumatic diseases concerns non-Hodgkin's lymphoma [44, 45], a cancer that had a significantly higher incidence in our study group than that in the general population.

Interestingly, the presence of lymphoma-like T cell infiltration involving both the spleen and liver has been reported in the homozygous dnTGFβRII IL-$6^{-/-}$ mice. Splenomegaly, hepatomegaly, and liver dysfunction were observed in homozygous dnTGFβRII IL-$6^{-/-}$ mice between 10 weeks and 10 months of age associated with a predominant infiltration of CD4(−)CD8(−)TCRβ(+)NK1.1(+) or CD8(+)TCRβ(+)NK1.1(−) T cell subsets [46]. The

Table 6 Cause of death in series in PBC patients

Author	Year	Country	No. of PBC patients	Mortality for liver disease (%)	Mortality for EM (%)	Mortality for other causes (%)
Myszor M [60]	1990	UK	194	81	N.A.	N.A.
Danielsson A [61]	1990	Sweden	111	15.3	0.9	6.3
Kim WR [62]	2000	USA	46	69.2	15.3	15.3
Sood S [63]	2004	Australia	249	23.6	N.A.	N.A.
Pla X [64]	2007	Spain	87	40	0	60
Wong RK [65]	2008	Singapore	34	100	0	0
Kubota J [66]	2009	Japan	308	41.6	33.3	25
Floreani A [67]	2011	Italy	327	74.9	1.12	6.2
Baldursdottir TR [68]	2012	Iceland	168	30	N.A.	N.A.

N.A. not available

dnTGFβRII mice are an animal model of PBC [47, 48]. It exhibits several major serological and histological characteristics of human PBC: (1) They are 100 % AMA positive with autoantibodies directed against the major mitochondrial autoantigens in human PBC including PDC-E2, BCOADC-E2, and OGDC-E2. (2) Their liver and serum cytokine levels reflect a Th1 profile. (3) The liver histology of dnTGFβRII mice manifests lymphoid cell infiltration in the portal tracts of mice including $CD4^+$, $CD8^+$, and $CD19^+$ cells as in human PBC. This is accompanied by bile duct injury in 25–50 % of mice up to 22 weeks of age [49]. (5) They developed ANAs to gp210 and Sp100 as in patients with PBC [50].

The presence of lymphoma-like T cell infiltration in the dnTGFβRII IL-$6^{-/-}$ mice implicates the underlying contribution of cytokines in malignancies and PBC. The complexity of the IL-12/IL-23 cytokine milieu in autoimmunity in dnTGFβRII mice was dissected by generating a series of cytokine knockouts with the dnTGFβRII mice. These include IFNγ$^{-/-}$, IL-12p35$^{-/-}$, IL-12/IL-23p40$^{-/-}$, IL-23p19$^{-/-}$, and IL-17A$^{-/-}$ [51–53]. Comprehensive analysis of multiple immunological parameters, cytokine profiles, AMA, and liver pathology in these constructs indicated that IL-12/Th1 pathway is essential for biliary disease pathogenesis, whereas the IL-23/Th17 pathway mediates colitis. To further determine the role of B cells in tissue pathology in dnTGFβRII mice, young (4–6 weeks) and old (20–22 weeks) dnTGFβRII mice were injected intraperitoneally with anti-mouse CD20 monoclonal antibody every 2 weeks, and their disease phenotype was compared with that of the control antibody-treated mice [41]. Treatment of young dnTGFβRII mice had a fully depleted serum AMAs, a lower incidence of liver inflammation, and fewer number of activated hepatic $CD8^+$ T cells. In contrast, anti-CD20 treatment dnTGFβRII mice with established disease was ineffective, suggesting that B cells play both a positive and negative regulatory role in the pathogenesis of PBC [54].

To examine the role of $CD4^+$ and $CD8^+$ T cells in liver pathology in this mice model of PBC, adoptive transfer studies were conducted by transferring dnTGFβRII mice derived splenic $CD4^+$ and/or $CD8^+$ T cells into Rag1$^{-/-}$ recipients. Transfer of dnTGFβRII derived $CD8^+$ T cells into Rag1$^{-/-}$ recipients resulted in liver specific autoimmunity, whereas $CD4^+$ T cell transfer led to colitis, indicating that $CD8^+$ T cells are the primary contributors for bile duct destruction in this model [55]. Recently, Tanaka et al. reported that adoptive transfer of Tregs from C57BL/6 mice significantly reduced the pathology of autoimmune cholangitis in dnTGFβRII mice, including decreased portal inflammation and bile duct damage as well as downregulation of the secondary inflammatory response. This study highlights the therapeutic potential of wild-type CD4+ Foxp3+ Tregs in reducing the excessive T cell responses of autoimmune cholangitis and the significance for the potential immunotherapy of PBC [56]. The requirement of antigen specificity of clonal CD8+ T cells and defective dnTGFβ signaling in the liver pathology was subsequently demonstrated [57].

The most important finding of our comprehensive study on EMs in PBC is that survival was similar in PBC patients with and without EM, and these patients' actual survival was much the same as the one predicted by the Mayo model. This prompts several considerations. First of all, the presentation and natural history of PBC have changed over the last 20 years: Most cases are diagnosed in an early stage nowadays, when the patients are still asymptomatic [58]. They are often identified when routine checkups reveal higher than normal serum alkaline phosphatase, GGT or total serum cholesterol levels, or on biochemical or serological investigations when an associated disease, such as scleroderma, comes to light. PBC has thus become a mild disease with a very long natural history. Second, the most important cause of death in PBC patients is related to liver disease, including HCC in end-stage disease (Table 6). A search on the outcome of PBC patients in published cohorts, in terms of the causes of death, revealed that mortality rate due to EM was low, ranging from 0.9 to 33.3 %, and the higher rates were only reached for elderly PBC groups, whereas mortality among younger patients was due mainly to the complications of portal hypertension.

In conclusion, the incidence of EM in patients with PBC is similar in Italy and Spain and no different from their incidence in the general population. Advanced histological stage and extrahepatic autoimmunity emerged as risk factors significantly associated with EM in PBC. The onset of EM during a patient's follow-up for PBC did not influence the natural history of their liver disease. Ultimately, further data such as that obtained in this study will influence the clinical management of patients with autoimmune biliary disease [59].

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