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Geoepidemiology, clinical manifestations and outcome of primary biliary cholangitis in Greece

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ABSTRACT

Background & aims: Primary biliary cholangitis (PBC) is a disease with rising prevalence and considerable geographical variation. To describe the prevalence, spatial and time distribution, baseline characteristics, response to treatment, outcome and the validity of GLOBE score in a large cohort of Greek PBC patients as an independent validation of this score has not been done so far.

Methods: The last 16 years, 482 PBC patients (86.5% females) were evaluated and analysed retrospectively, using a prospectively collected database. Special attention was paid to the assessment of treatment response according to GLOBE score.

Results: Age at initial evaluation was 56.3 ± 13.7 years. Among 432 Thessaly residents, prevalence was 582/million (non-homogeneous distribution). Nineteen districts showed a prevalence >800/million. Symptomatic disease onset could be identified in 91 patients, with a significant peak during spring ($P = 0.03$). At diagnosis, 43.6% were asymptomatic and 16.2% cirrhotic. Male sex ($P = 0.02$), older age ($P < 0.001$), alcohol consumption ($P < 0.01$) and concomitant liver disease ($P < 0.001$) were negative prognostic factors for cirrhosis. During a median [interquartile range, range] follow-up of 5.1 (7.8, 15.7) years, 62 patients died or underwent liver transplantation. Patients with GLOBE score > 0.30 had significantly worse prognosis ($P < 0.001$) with 5-, 10-, and 15-year survival rates of 84%, 50% and 42%.

Conclusions: There is increased PBC prevalence in Thessaly with remarkable geographic clustering and seasonal variability. PBC is diagnosed at early stages although males had a more advanced disease. GLOBE score applies perfectly in Greek patients and this will likely help detecting patients that may benefit from new therapies.

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1. Introduction

Primary biliary cholangitis (PBC) previously known as primary biliary cirrhosis is an autoimmune cholestatic liver disease characterized by a progressive inflammatory destruction of the small intrahepatic bile ducts with portal inflammation, progressive fibrosis, leading to cirrhosis and subsequent liver failure [1,2].

It is considered a model of autoimmune disease with specific autoantibodies, female predominance and frequent coexistence with other autoimmune conditions [1–3]. The serologic hallmark of PBC is the detection of anti-mitochondrial antibodies (AMA), directed against the E2-subunit of pyruvate dehydrogenase multi-enzyme complex located in the inner membrane of mitochondria [3,4], though several other antibodies either disease-specific or not have been detected [3,5–7]. AMA are detectable in 85–95% of patients with PBC and frequently occur long before clinical signs or symptoms appear [1–3,8].

Clinical presentation at the time of diagnosis varies from asymptomatic with normal or abnormal biochemical tests, to symptomatic, or finally to advanced liver disease. Presenting symptoms are frequently fatigue, pruritus and arthralgias in the absence of other signs of liver disease. It is now well-known, that more patients with asymptomatic PBC are being diagnosed thanks to the routine use of biochemical screening, increased medical awareness of the disease, and improvements in AMA testing [2,3,9].

The etiology of the disease remains unknown, although it is accepted that its pathogenesis has a multifactorial basis. The available evidence indicates that environmental factors may trigger PBC in genetically predisposed individuals. Genetic predisposition is supported by high concordance observed among monozygotic twins [10], high level of PBC aggregates in families [11], and significant associations with specific gene polymorphisms [12–14]. However, the concordance rate of only 63% in monozygotic twins along with the worldwide geographical heterogeneity regarding its prevalence and incidence give further support

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to the environmental impact in the development of disease clinical phenotype [10,15]. Indeed, differences noticed in PBC prevalence have been reported [16–18] and may reflect differences in exposure to environmental factors, although the determination as well the quantification of environmental influences is very difficult to be clearly defined [19]. In this context, it has been reported that environmental risk may be attributable either to infectious agents (viruses and bacteria) or xenobiotics [20,21].

Several studies have investigated the epidemiology of PBC worldwide and population-based studies, attempting to estimate the prevalence and the incidence of PBC, have introduced the concept of geoepidemiology of the disease [22], taking into account that prevalence rates among studies range from 19.1 to 402 per 1 million inhabitants and they are markedly increasing over time [23]. However, so far only one study from Crete island has described the characteristics of PBC in Greece [24]. Therefore, we conducted a retrospective analysis of prospectively collected data of a large cohort of PBC patients in order to evaluate PBC characteristics in the mainland (Central Greece, Thessaly) and to describe the spatial and time distribution of the disease in this area along with the validity of the GLOBE score as an independent validation of this score has not been done so far in PBC patients.

2. Materials and methods

2.1. Patients

A retrospective analysis of a prospectively collected database of all 482 consecutive patients with well-defined PBC, seen at the Department of Medicine, Medical School, University of Thessaly, Larissa, Greece between 2000 and 2015 was performed. Patients were followed at our outpatient clinic for a median [interquartile range (IQR), range] period of 5.1 (7.8, 15.7) years.

According to internationally accepted criteria and to our reports, PBC diagnosis was based on the presence of at least two of the following criteria: [7,25–27] (a) AMA-positivity, (b) elevated cholestatic enzymes for >6 months and (c) histological lesions of PBC.

In order to assess for possible differences in PBC distribution across Thessaly region, we have recorded the place of residence of patients. Thessaly is one of the thirteen peripheries of Greece and covers the major part of Central Greece. The prefectures of Larissa (capital city of Larissa), Magnesia (capital city of Volos), Trikala (capital city of Trikala) and Karditsa (capital city of Karditsa) and the islands of Sporades constitute Thessaly. According to the most 2 recent censuses (2001 and 2011) the population remained stable during the study period (approximately 750,000 people; 6.8% of the national population). Of note, the population of Thessaly is considered homogeneous, mainly of Caucasian origin and with a small proportion of immigrants. Residents of Thessaly were 439 out of 482 patients (91.1%) at the time point of initial evaluation.

Medical records of patients were retrieved and reviewed and data concerning patients' demographics, educational level, lifestyle, medical and family history at the period prior to PBC diagnosis, clinical and laboratory parameters at the time-point of initial evaluation, as well as outcome and treatment response during follow-up were collected. Patients with symptoms attributable to PBC, like pruritus, fatigue in the absence of other identifiable causes, abdominal pain in the right upper quadrant not attributable to another etiology, jaundice, arthralgia, sicca syndrome and major complications of end-stage liver disease (ascites, gastrointestinal bleeding due to varices, hepatic encephalopathy) were defined as symptomatic. The presence of at least one of extrahepatic autoimmune conditions was defined as “concurrence of other autoimmune diseases”. Baseline levels of alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, prothrombin time (PT), albumin, serum immunoglobulin M (IgM) were determined using standard techniques. The PBC Mayo Risk Score was calculated for all patients at diagnosis [28]. Histological staging was carried out according to Ludwig's classification [29]. For patients without an available biopsy, diagnosis of cirrhosis was based on ultrasonography (nodules in liver parenchyma, spleen > 12 cm, portal vein > 16 mm), and/or endoscopic findings of cirrhosis (varices, portal gastropathy), and/or clinical findings of decompensation. Treatment response was assessed according to the GLOBE score (≤0.30 for responders and >0.30 for non-responders) in patients treated for at least 1-year with ursodeoxycholic acid (UDCA) [30]. In brief, according to the original study by Lammers et al., a GLOBE score above the threshold of 0.30 one year after the initiation of UDCA treatment characterizes PBC patients with diminished survival compared to the general population [30]. The performance of the GLOBE score was compared to the UK-PBC risk scores [31].

2.2. Autoantibodies detection

AMA (positive titer ≥ 1/40) were initially detected by indirect immunofluorescence on 5 μm fresh frozen sections of in-house rodent multiorgan (kidney, liver and stomach) tissue substrates, as we described previously [3,7,26,32,33]. Presence of AMA was confirmed by Western blot using in-house mitochondrial subfraction of rat livers, or by enhanced performance M2 ELISA (M2 EP (MIT3) ELISA, QUANTA Lite (R), INOVA Diagnostics) [3,7,34]. PBC-specific antinuclear antibodies (ANA) namely, antibodies against nuclear pore membrane glycoprotein 210 (anti-gp210) and antibodies against nuclear sp100 protein (anti-sp100) were detected by commercial ELISAs according to the manufacturers' instructions (INOVA Diagnostics) [5,7].

2.3. Ethics approval

The study was conducted in accordance with the protocol and the principles of the Declaration of Helsinki. The protocol was reviewed and approved by the Ethics Committee of Thessaly University, Medical School.

2.4. Statistical analysis

Data were entered and analysed using SPSS and Epi-Info 2002 software. Kolmogorov-Smirnov test was used to assess the normality of the distribution of variables. Normally distributed values are expressed as mean ± standard deviation (SD), while non-normally distributed as median (IQR). Data were analysed by *t*-test, Mann-Whitney *U* test, χ^2 test (two by two with Yate's correction), Fischer's exact test, Cox regression analysis, binary logistic regression model, multiple Cox regression model, area under receiver operating characteristic curve (AUC) and Delong's test where applicable. A seasonal effect in incidence assessed by calculating individual χ^2 tests for each season comparing observed to expected values. The expected values were determined by distributing the patients equally among the 12 months of a year, assuming that no seasonal effect exists. Two-sided *P*-values <0.05 were considered as statistically significant.

3. Results

Demographic and clinical characteristics of patients at the time point of initial evaluation are shown in Table 1. There were 417 (86.5%) women (female/male ratio: 6.4:1). The age at diagnosis was 56.3 ± 13.7 years in total population, with no differences between females and males (56.3 ± 13.3 vs. 56.5 ± 15.7 years, respectively; $P = 0.883$; Supplementary Fig. 1). AMA were detected in 465/482 (96.5%) patients by using any of the three described methods (AMA, ELISA, western blot), while ANA-PBC-specific antibodies (anti-sp100 and/or anti-gp210) were found in 66/482 (13.7%); anti-sp100, $n = 48$ (10%) and anti-gp210, $n = 26$ (5.4%). Of note, AMA negative patients were significantly more frequent in ANA-PBC-specific positive patients (6/66; 9.1%) compared to those having a negative test for ANA-PBC-specific antibodies (11/416; 2.6%; $P < 0.02$). No differences were found regarding

Table 1
Demographic and clinical baseline characteristics of PBC patients (total group, $n = 482$ and residents of Thessaly, $n = 439$).

	Total group	Residents of Thessaly
Sex, female/male, n (%)	417/65 (86.5%/13.5%)	382/57 (87%/13%)
Age, mean ± SD, years	56.3 ± 13.7	56.8 ± 13.7
Educational level[a] (null or primary/secondary/university), n (%)	225/93/91 (55%/23%/22%)	221/83/81 (57%/22%/21%)
Alcohol consumption		
Female (>20 g/day), n (%)	8 (1.9%)	7 (1.8%)
Male (>40 g/day), n (%)	19 (29.2%)	15 (26.3%)
Presence of symptoms, yes/no, n (%)	272/210 (56.4%/43.6%)	245/194 (55.8%/44.2%)
Co-incidence of other liver disease, yes/no, n (%)	30[b]/452 (6.2%/93.8%)	29[#]/410 (6.6%/93.4%)
ALP, median (IQR), UNL: 104 U/L	115 (81)	115 (80)
γ-GT, median (IQR), UNL: 37 U/L	84 (112)	84 (106)
AST, median (IQR), UNL: 40 U/L	36 (31)	36 (31)
ALT, median (IQR), UNL: 40 U/L	43 (42)	42 (44)
Bilirubin, median (IQR), UNL: 1.1 mg/dL	0.7 (0.4)	0.7 (0.5)
PT, median (IQR), *sec*	12.5 (1.6)	12.5 (1.7)
Albumin, median (IQR), normal range 3.5–5.2 g/dL	4.3 (0.6)	4.3 (0.6)
IgM, median (IQR), UNL: 200 mg/dL	171 (192)	169 (189)
Mayo Risk Score, median (IQR)	4.04 (1.72)	4.09 (1.79)
AMA, pos/neg, n (%)	465/17 (96.5%/3.5%)	423/16 (96.4%/3.6%)
Anti-gp210, pos/neg, n (%)	26/456 (5.4%/94.6%)	23/416 (5.2%/94.8%)
Anti-sp100, pos/neg, n (%)	48/434 (10%/90%)	40/399 (9.1%/90.9%)
Biopsy[c] stage (%), 0-I-II/III-IV	232/49 (82.6%/17.4%)	213/46 (82.2%/17.8%)
Cirrhotic, yes/no, n (%)	78/404 (16.2%/83.8%)	73/366 (16.6%/83.4%)

Abbreviations are same as in the text. n = number of patients in each respective group. UNL = upper normal limit.

[a] Educational level was defined in 409 out of 482 patients in the total group of patients and in 385 out of 439 residents of Thessaly.

[b] 17 patients with chronic viral hepatitis B or C, 11 with variant form of PBC – autoimmune hepatitis, 1 with hemochromatosis and 1 with nodular regenerative hyperplasia ([#]one out of 11 patients with variant form of PBC – autoimmune hepatitis lived outside Thessaly region).

[c] Liver biopsy at baseline was performed in 281 patients of the total group and in 259 patients with residency in Thessaly.

the demographic and clinical baseline characteristics between the total group of patients ($n = 482$) and PBC patients with residency in Thessaly ($n = 439$) (Table 1).

3.1. Prevalence and seasonal variation of PBC

Among 439 residents of Thessaly, 241 (54.9%) were living in the prefecture of Larissa, 74 (16.9%) in Magnesia, 71 (16.2%) in Trikala and 53 (12.1%) in Karditsa. All 439 patients had spent most of their lives in Thessaly region. Residency of PBC patients was equally distributed among urban (222/439, 50.6%) and rural (217/439, 49.4%) areas, while the proportion of residents of rural areas exceeded that of urban areas in the general population (45.2% in urban and 54.8% in rural areas, $P < 0.03$, census data 2011). The estimated period prevalence of PBC in Thessaly was 582/1 million inhabitants (number of cases diagnosed during the study period), with considerable variation among different areas. Supplementary Fig. 2 shows the geographic distribution of PBC patients that reside in Thessaly. In 19 municipalities, we identified prevalence higher than 800 cases/1 million inhabitants.

Ninety-one out of 482 patients (19%) were able to accurately define the onset of symptoms within a particular month of the year. Stated symptomatic onset clustered in the spring, with overall χ^2 heterogeneity test indicating statistically significant evidence for deviation from the uniform distribution ($P = 0.03$) (Fig. 1). Similar findings were found in 84 patients out of 439 (19.1%) residents of Thessaly, that could determine the onset of symptoms, showing a significant clustering during spring ($P < 0.02$).

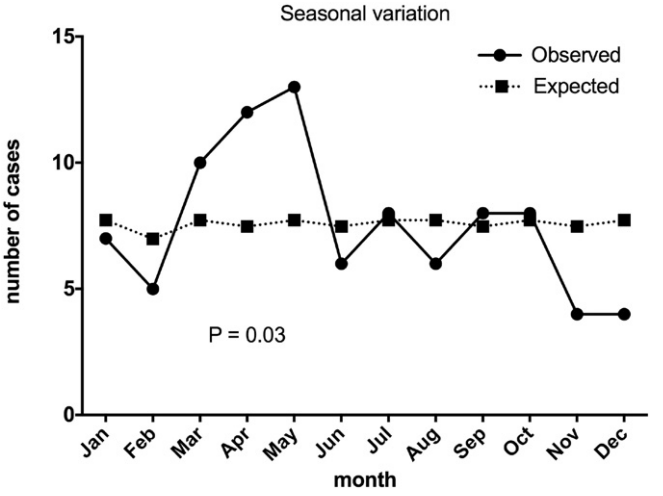

Fig. 1. Symptomatic onset clustering in the spring. The determination of the expected values in the 91 patients who were able to accurately define the onset of symptoms within a particular month of the year was done by distributing the cases equally among 12 months, assuming that no seasonal effect exists.

3.2. Risk factors associated with PBC

Supplementary Table shows the frequencies of known risk factors associated with PBC in the total group of patients ($n = 482$) and in PBC patients who were residents of Thessaly ($n = 439$). Eighteen out of 482 patients had first degree relatives with PBC, resulting in a known family history of 3.7%. At least one concurrent autoimmune disease was observed in 137/482 patients (28.4%), whereas 32/482 (6.6%) suffered from more than one autoimmune diseases. The most common autoimmune disease was autoimmune thyroid disease ($n = 65$, 13.5%) with Hashimoto thyroiditis in 62/482 (12.9%). No difference was observed between females and males regarding the prevalence of concurrent autoimmune diseases (123/417, 29.5% vs. 14/65, 21.5%, respectively, $P = 0.240$). Previous or active smoking was reported by 128 (26.6%) patients and hormone replacement treatment by 5 (1%) patients. Fifty-three (11%) patients reported a history of urinary tract infections, with known causative agent in 22 (*Escherichia coli* $n = 19$, Proteus $n = 1$, *Escherichia coli*/Klebsiella spp. $n = 1$, *Escherichia coli*/Mycoplasma/Ureoplasma $n = 1$). Thirty-seven patients (7.7%) reported a history of cancer, breast cancer being the commonest neoplasia in female patients (15/424, 3.5% of the female population). The distribution of malignancies among PBC patients is shown in Supplementary Table. No significant correlation was found between sex and risk factors, apart from smoking that was more frequent in males (37/65, 56.9% vs. 91/417, 21.8%, $P < 0.001$).

Educational level was identified in 409 out of 482 patients with a predominance of patients with null or primary level (225/409, 55%) compared to patients with secondary (93/409, 23%) or university education (91/409, 22%; $P < 0.001$). Compared to the general population (from the more recent census data, 2011), lower educational level was more frequent in PBC patients (55% vs 46%, $P < 0.001$; data not shown). Alcohol consumption (>40 g/day for males, >20 g/day for females) was significantly more frequent in males (19/65, 29.2% vs. 8/417, 1.9%, $P < 0.001$). No differences were found regarding the risk factors associated with PBC between the total group of patients ($n = 482$) and the PBC patients with residency in Thessaly ($n = 439$) (Supplementary Table).

3.3. Symptoms related to PBC

In the total group of patients ($n = 482$), 272 (56.4%) were symptomatic at baseline visit (77/482, 16% reported more than one symptom); 125 (25.9%) reported itching, 72 (14.9%) sicca syndrome, 57 (11.8%) fatigue, 54 (11.2%) musculoskeletal pain, 42 (8.7%) abdominal pain, 7 (1.5%) rash (erythema nodosum $n = 1$, Henoch-Schonlein purpura $n = 3$, vitiligo $n = 3$), while 12 (2.5%) had symptoms due to end-stage liver disease. Similarly, in the group of patients with residency in Thessaly ($n = 439$), 245 patients (55.8%) were symptomatic at initial evaluation, without differences regarding to the type of symptoms when compared to the total patient population ($n = 482$).

Symptoms appeared more frequently in females (245/417, 58.8% vs. 27/65, 41.5%, $P = 0.014$), but there was a statistical trend towards males presenting more frequently with end-stage liver related symptoms (4/65, 6.2%) compared to females (8/409, 1.9%, $P = 0.06$). In parallel, in the subgroup of patients with residency in Thessaly, females were more frequently symptomatic (222/382, 58.1% vs. 23/57, 40.4%; $P < 0.02$), with males presenting more commonly with end-stage liver related symptoms (4/57, 7% vs. 7/382, 1.8%, $P < 0.05$).

No significant difference was noticed regarding the age and presence of symptoms at initial presentation (57 ± 13.8 for symptomatic vs. 55.5 ± 13.4 years for asymptomatic patients, $P = 0.236$ in the total group of patients and 57.4 ± 13.9 vs. 56.1 ± 13.4 years, $P = 0.36$ in the group of Thessaly residents with PBC).

3.4. Severity of liver disease

At the time of diagnosis, 78/482 patients (16.2%) had cirrhosis, while 2/482 (0.4%) diagnosed with hepatocellular carcinoma (HCC) at initial evaluation. Univariate analysis showed that cirrhosis was more frequent in males (22/65, 33.8% vs. 56/417, 13.4%, $P < 0.001$), older patients (67.6 ± 11 years in cirrhotic vs. 54.1 ± 13 years in non-cirrhotics; $P < 0.001$), patients with co-incidence of other liver disease (12/30, 40% vs. 66/452, 14.6%; $P = 0.001$), significant alcohol consumption (12/27, 44.4% vs. 66/455, 14.5%; $P < 0.001$) and lower educational level (46/225, 20.4% vs. 15/184, 8.2%; $P = 0.001$).

Binary logistic regression analysis in the total group of patients ($n = 482$) revealed that male sex ($P = 0.02$), older age ($P < 0.001$), alcohol consumption ($P < 0.01$) and presence of other liver disease ($P < 0.001$) remained independent significant risk factors for the presence of cirrhosis (Table 2). Similar findings revealed from the analysis in the group of patients who were residents of Thessaly ($n = 439$), with male sex ($P = 0.03$), older age ($P < 0.001$), alcohol consumption ($P < 0.01$) and co-incidence of other liver disease ($P = 0.001$) constituting negative prognostic factors for cirrhosis presence.

A second multivariate model (data not shown) incorporating the educational level (available in 409 patients from the whole group), revealed that older age (OR = 1.096, 95% CI: 1.026–1.132; $P < 0.001$), concomitant other liver disease (OR = 3.726, 95% CI: 1.387–10.007; $P < 0.01$), alcohol consumption (OR = 5.022, 95% CI: 1.588–15.884; $P < 0.01$) and male sex (OR = 2.328, 95% CI: 0.985–5.504; $P = 0.05$) were independent risk factors for the presence of cirrhosis at the time of diagnosis. Identical results were found in the subgroup of patients resided in Thessaly region. Of the 281 patients from the total group and 259 patients from the group of residents of Thessaly with available histology at baseline, 232/281 (82.6%) and 213/259 (82.2%) had "early disease" (stage 0-I-II), while 49/281 (17.4%) and 46/259 (17.8%) had "advanced disease" (stage III-IV).

Table 2
Binary logistic regression analysis of baseline factors associated with cirrhosis at initial evaluation of patients ($n = 482$).

Factors	Adjusted OR	95% CI	Adjusted *P*-value
Sex (male)	2.437	1.135–5.232	0.02
Age	1.106	1.076–1.136	<0.001
Co-incidence of other liver disease	5.108	2.098–12.434	<0.001
Alcohol consumption	4.851	1.697–13.867	<0.01

3.5. Response to treatment

The majority of patients received UDCA as standard of care (13–15 mg/kg/day) [25]. GLOBE score was assessed in 271 patients that were treatment-naïve at baseline visit, had no evidence of other concurrent liver disease and had available parameters 12 months after treatment. Seventy-four patients (74/271, 27.3%) characterized as non-responders with a GLOBE score > 0.30, while 197/271 (72.7%) as responders (score ≤ 0.30). Non-responders were characterized by lower educational level ($P < 0.001$), increased IgM ($P < 0.001$), anti-gp210 positivity ($P < 0.05$), advanced histological stage ($P < 0.001$) and presence of cirrhosis ($P < 0.001$) at baseline visit (Table 3). Multivariate analysis showed that cirrhosis (OR = 22.312, 95% CI: 5.988–83.137, $P < 0.001$) and lower educational level (OR = 2.993, 95% CI: 1.441–6.217, $P < 0.01$) were negative predictive factors for response to treatment. Indistinguishably, after multivariate analysis in the subgroup of 247 PBC patients (treatment-naïve without other liver disease) that were residents of Thessaly with available GLOBE score, cirrhosis (OR = 20.163, 95% CI: 5.414–75.096, $P < 0.001$) and lower educational level (OR = 2.883, 95% CI: 1.340–5.989, $P < 0.01$) correlated negatively with response to treatment.

3.6. Outcome

During a median follow-up of 5.1 years (IQR, 7.8 years), 62 patients reached a clinical end point; 60 patients died (38 due to liver-related causes) and 2 patients underwent orthotopic liver transplantation (OLT). Patients who died from liver-related causes were older (median 71 years, IQR 11 years). In more detail, the reason of no OLT in this group was the increased age (>70 years) in 24, severe co-morbidities in 6 and continuing alcohol abuse in 3 (the remaining 5 patients died awaiting transplant). Among 2 patients with HCC diagnosed at baseline, one patient died during follow-up, while the other one was alive at the most recent visit. Another two patients developed HCC during follow-up and subsequently died. From 40 liver-related events (OLT or death), 6 occurred in patients with concurrent liver diseases (1 hepatitis C, 2 hepatitis B, 3 autoimmune hepatitis). Liver-related mortality was higher in males (16/60, 26.7% vs. 24/400, 6%, $P < 0.001$). Multiple Cox regression analysis (Table 4) showed that negative prognostic factors for liver-related events were significant alcohol consumption (HR = 4.159, 95% CI: 1.031–16.777, $P < 0.05$), increased bilirubin (HR = 1.901, 95% CI: 1.342–2.694, $P < 0.001$), presence of cirrhosis at baseline (HR = 14.803, 95% CI: 3.578–61.244, $P < 0.001$) and advanced age at baseline (HR = 1.113, 95% CI 1.058–1.172, $P < 0.001$). In the subgroup of 439 patients who lived in Thessaly, during a median follow-up of 5.5 (7.7) years, 37 died from liver-related causes and 22 from other causes. In the same manner, multivariate model revealed alcohol (HR = 4.963, 95% CI: 1.211–20.347, $P < 0.03$), high bilirubin levels (HR = 1.974, 95% CI:1.357–2.873, $P < 0.001$), cirrhosis (HR = 15.227, 95% CI: 3.717–62.378, $P < 0.001$) and older age (HR = 1.121, 95% CI 1.059–1.186, $P < 0.001$) as negative prognostic factors for liver-related events.

GLOBE score > 0.30 was associated with significantly diminished transplant-free survival compared to patients with score ≤ 0.30 (HR = 58.83, 95% CI: 7.739–447.223, $P < 0.001$ in the total group and HR = 54.32, 95% CI: 7.143–413.071, $P < 0.001$ in Thessaly residents). The 5-, 10-, and 15-year survival or transplant-free rates for non-responders were 84%, 50%, and 42%, respectively for both groups (Fig. 2). Multiple Cox regression analysis showed that GLOBE score > 0.30 remained an independent negative prognostic factor (HR = 17.159, 95% CI: 1.982–148.53, $P = 0.01$ in the total group and HR = 15.841, 95% CI: 1.833–136.92, $P < 0.02$ in Thessaly residents) after adjustment for the presence of cirrhosis at baseline and alcohol consumption (data not shown).

Table 3
Comparison of baseline characteristics between responders and non-responders to UDCA according to GLOBE score ($n = 271$).

	Responders ($n = 197$) GLOBE score ≤ 0.30	Non-responders ($n = 74$) GLOBE score > 0.30	*P* value
Female, n (%)	175 (88.8)	64 (86.5)	0.747
Alcohol consumption	10 (5.1%)	5 (6.8%)	0.562
Educational level[a], n (%)			<0.001
Null or primary	74 (42.3)	46 (71.9)	
Secondary or university	101 (57.7)	18 (28.1)	
Presence of symptoms, n (%)	118 (59.9)	48 (64.9)	0.543
IgM, median (IQR), UNL: 200 mg/dL	153 (159)	222 (279)	0.001
AMA positivity, n (%)	188 (95.4)	72 (97.3)	0.733
Anti-gp210 positivity, n (%)	7 (3.6)	8 (10.8)	<0.05
Anti-sp100 positivity, n (%)	19 (9.6)	10 (13.5)	0.486
Biopsy stage[b], n (%)			<0.001
0-I-II	144 (92.9)	28 (63.6)	
III-IV	11 (7.1)	16 (36.4)	
Presence of cirrhosis, n (%)	5 (2.5)	25 (33.8)	<0.001

Abbreviations are same as in the text. n = number of patients in each respective group.
[a] Educational level was defined in 239 out of 271 patients.
[b] Liver biopsy was available in 199 patients.

GLOBE score had a high discriminative ability with AUC (95% CI) being 0.844 (0.669–1.000), 0.892 (0.799–0.984), and 0.892 (0.803–0.981) for 5-, 10-, and 15-year liver-transplant or liver-related-death free survival, which were comparable to the respective UK-PBC risk scores regarding the 5-year survival [0.765 (0.599–0.930), $P = 0.337$], and superior regarding the 10-year survival [0.785 (0.677–0.894), $P < 0.05$, and 15-year survival [0.774 (0.668–0.880), $P < 0.02$] (Fig. 3). Similarly, in the group of patients with residence in Thessaly, AUC (95% CI) for the GLOBE score were 0.840 (0.665–1.000), 0.888 (0.795–0.981), 0.888 (0.800–0.977) for 5-, 10-, and 15-year liver-transplant or liver-related-death free survival, whereas for the respective UK-PBC risk scores were 0.768 (0.605–0.931), 0.790 (0.683–0.897) and 0.780 (0.675–0.884) ($P = 0.373$, $P = 0.05$, $P < 0.03$ for each comparison).

4. Discussion

We report herein the largest study ever performed in Greece on PBC in a well-defined cohort of patients. An increased prevalence of PBC was found with remarkable geographic clustering, as well as a seasonal variability of the symptomatic disease onset with the peak of incidence during spring. Almost half of our patients were asymptomatic at baseline visit, with the majority of them having an early histological stage. As expected, male patients were a minority compared to females (female/male ratio: 6.4/1) but they presented more frequently with end-stage liver disease and relevant symptoms. Finally, the GLOBE score appears to perform well in the Greek population as prediction tool of transplant-free survival of UDCA-treated patient. These findings were almost identical either in the total group of patients ($n = 482$) or the subgroup of patients resided in Thessaly region ($n = 439$).

Prevalence of PBC varies widely depending on geographical location [16,23]. Additionally, recent studies have shown increasing incidence and prevalence rates of the disease over time [23]. Our data strongly indicate that there is a high prevalence of PBC in Central Greece (582 cases/million inhabitants) with a clustering of PBC cases in 19 districts (prevalence >800 cases/million). In the only study on 245 PBC cases from Greece [24], a prevalence of 365 cases/million was reported in Crete island, with high-risk areas of disease occurrence located in the Eastern part of the island. Our reported prevalence is in line with previous studies with the highest prevalence reported so far [16,35]. A prevalence of 400/million is now considered average in areas of high medical awareness of the disease, availability of optimal autoantibody testing and clinical expertise. In line with this, our Department serves as the only referral centre for autoimmune liver diseases in Central Greece, though due to the structure of the existing healthcare system in our country it also operates as primary and secondary healthcare facility. As a result, we believe that we have identified the majority of PBC patients in the area, who have sought medical advice, taking into account that all regional hospitals as well as private doctors/physicians refer to our department almost all diagnosed PBC patients as well as those presenting with deranged liver function tests. Still, we cannot exclude the possibility of having missed PBC cases, considering that no strict case-finding methods were applied and differences regarding the availability of access to care among the different areas though highly unlike for our healthcare system, could exist. For instance, a point that may support further underreporting issues is the relative lack of cases from the peripheral districts of west part of Thessaly, as shown in Supplementary Fig. 2. However, this could be attributed to the referral of patients

Table 4
Multiple Cox regression analysis of the risk factors at baseline associated with liver-related events during follow-up ($n = 460$[a]).

Variables	Adjusted HR	95% CI	P
Male sex	0.464	0.135–1.595	0.223
Age	1.113	1.058–1.172	<0.001
Alcohol consumption	4.159	1.031–16.777	0.045
Bilirubin	1.901	1.342–2.694	<0.001
PT	1.141	0.948–1.374	0.163
Albumin	0.767	0.381–1.543	0.456
IgM	1.000	0.999–1.002	0.681
Presence of cirrhosis	14.803	3.578–61.244	<0.001

Abbreviations are same as in the text.
[a] Patients died from non-liver related causes ($n = 22$) were excluded from multivariate analysis.

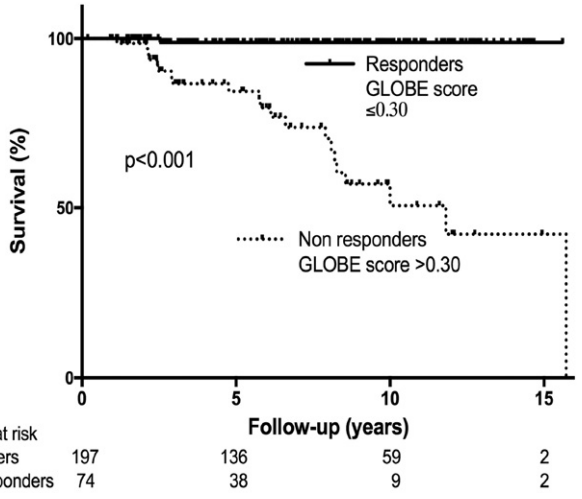

Fig. 2. Diminished survival in patients with GLOBE score > 0.30 (HR = 58.83, 95% CI 7.739–447.223; $P < 0.001$). The 5-, 10-, and 15-year survival or transplant-free rates for non-responders were 84%, 50%, and 42%, respectively.

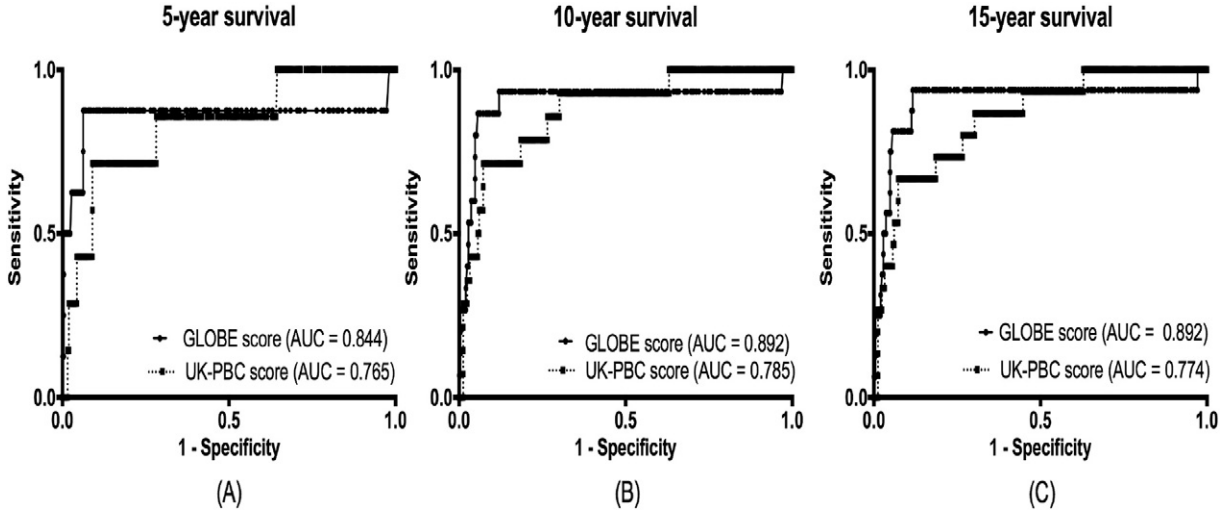

Fig. 3. Receiver operating characteristic (ROC) curves for the prediction of liver-transplant or liver-related-death free survival according to the GLOBE and UK-PBC risk scores at 5 (A), 10 (B) and 15-years (C).

from those geographical areas to the neighboring University Hospital of Ioannina (located in West Greece outside Thessaly region), and not to access barriers.

Significant differences in the reported prevalence rates among studies may reflect disparities in exposure to environmental factors [16–18]. Among environmental factors, pollution related with coal mining activities has been proposed as a triggering agent [16]. Of note, the underground of five described areas in our study with increased prevalence (Sarantaporo, Olympos, Verdikousia, Elassona, Tirnavos) is rich in coal and nearby a thermal power station operates using coal (lignite) as source of energy. Triger et al. [36] showed an apparent clustering of cases in the city of Sheffield, where the prevalence in relation to one water reservoir appeared to be more than ten times that of the other reservoirs. Prince et al. [37] demonstrated also a non-random distribution of cases of PBC in the Northeast England, reflecting one or more environmental risk factors in PBC pathogenesis. In another study by Ala et al. [38], the prevalence of PBC patients listed for transplantation was increased near Superfund toxic waste sites in New York City, indicating environmental pollution with aromatic and halogenated hydrocarbons as potential triggering or contributing factors for PBC. In line with the abovementioned data, the risk in migrants was found to correlate with the risk of the population into which they move [18].

Our study provides also evidence of seasonal variation of the symptomatic disease onset. In particular, a highly statistically significant excess of reported disease onset during spring was found. Similar to our findings McNally et al. [39] reported a marked peak of PBC diagnosis in June. These findings support the hypothesis that a seasonally varying environmental factor, occurring close to disease onset, may trigger PBC development or transition from a latent stage to clinically overt disease at least in some patients. Indeed, our study could not favour the hypothesis of an infectious agent, because analysis of time-related confounding factors related to seasonal variations is not practically feasible, taking into account the complexity of candidate agents involved, such as infections and xenobiotics. A number of infectious agents such as *E. coli*, Mycobacteria, Novosphingobium aromaticovorans, Lactobacillus species, Chlamydia pneumoniae, Helicobacter pylori, human beta retrovirus and mouse mammary tumor virus have been associated with PBC, through molecular mimicry mechanisms leading to the breakdown of immune tolerance and subsequent induction of PBC [1,2]. History of urinary tract infections was reported by 11% of our patients, with *E. coli* as the dominant causative agent. Xenobiotics have also been implicated in the etiology of PBC and these agents need specific attention, as many environmental chemicals can modify hepatic or biliary cellular proteins to form neo-antigens [21,22]. The number of exogenous compounds potentially leading to modifications is enormous, from food preservatives and cosmetics to several pollutants [1,22]. Besides, atmospheric concentrations of pollutants are known to exhibit seasonal variation, providing support for a possible link with the onset of PBC at specific periods of the year [40].

Our large cohort study confirmed known risk factors associated with PBC including recurrent urinary tract infections, concomitant autoimmune diseases, smoking, estrogen replacement therapy and family history of PBC [11,41–43]. Of note, a history of malignancy was positive in 8% of our patients which is similar to that reported from another two Greek studies [44,45]. In addition, we found a predominance of patients with lower levels of education. We cannot exclude the possibility of environmental exposures having contributed to clustering of PBC cases among persons with socioeconomic deprivation, as has been suggested by McNally et al. in a well-defined PBC cohort in North East England [46]. Besides, lower educational level was associated with the presence of cirrhosis at baseline and negatively with the response to treatment. This may reflect probable socioeconomic disparities in health, with patients of lower socioeconomic status having a delay in diagnosis due to unawareness of liver diseases or restricted access in the health system, resulting in a more advanced disease at the time-point of diagnosis which in turn can lead to a decreased response to treatment.

From a clinical point of view, few studies have taken into account PBC in men. In this context, we were not able to detect any difference among risk factors compared to women apart from a higher percentage of smoking in males, which is particularly of interest, as smoking has been demonstrated as a potential predisposition and aggravating factor for PBC [41,42,47]. However, we observed significant differences between genders in terms of the presence of symptoms at baseline visit. Females reported more frequently symptoms, but males experienced more often symptoms associated with end-stage liver disease during the initial evaluation. Moreover, we found that male sex was an independent factor for the presence of cirrhosis at baseline visit even after the adjustment of age, presence of concomitant liver diseases and alcohol consumption. Muratori et al. [48] have shown a higher percentage of males in patients with advanced stage of liver disease, although this was not found to be statistically significant.

In two-thirds of our patients who reached a clinical end-point (death or OLT), the cause was related to liver disease, and only one-third died of extrahepatic causes. As expected, liver-related mortality was significantly associated with the presence of cirrhosis and levels of bilirubin at baseline visit, which is in line with the findings from a large Italian study [49]. In parallel with these findings is the very low incidence of HCC observed in our cohort as in association with the known low

frequency of HCC development in autoimmune liver diseases compared to viral or alcoholic liver diseases most of our patients were diagnosed at an early disease stage (only 16% were cirrhotic at initial presentation). One interesting finding from our multivariate analysis was that gender was not proved to be a predictive factor for survival and this happened due to differences noted in alcohol consumption between sexes, an aggravating factor that was not taken into account in previous studies [49,50].

Finally, in the present study, we evaluated for the first time the performance of GLOBE score in a large cohort of Greek PBC patients. GLOBE score comprises 5 simple, readily available and objective variables (age, bilirubin, albumin, ALP and platelet count) and is able to accurately stratify patients in high and low risk, predicting transplant-free survival of UDCA-treated PBC patients [30]. So far, this score has not been externally validated. Risk prediction for non-responder according to GLOBE score in our Greek population (5-, 10, and 15-year transplant-free survival rates of 84%, 50%, and 42%, respectively) was similar to that of the original data (79.7%, 57.4%, and 42.5% respectively) [30], suggesting an excellent applicability of this score in Greek patients. Moreover, comparison with the UK-PBC risk scores showed that the GLOBE score had a better discriminative ability to predict survival in Greek PBC patients.

In conclusion, we have described the geoepidemiology and seasonal variability of PBC in Greece, along with baseline characteristics, response to treatment and outcome in a large cohort of patients. Although this study was retrospective and not population-based, we strongly believe that the observed prevalence is reliable and among the highest reported so far, as our data was prospectively collected during the last 16 years for all PBC patients. Indeed, our data may suggest implication of environmental risk factors in the pathogenesis of the disease, although further epidemiological studies, especially in countries with national patient registries and accurate toxic waste site data will help to determine the impact of the environment on the development of such a complex disease like PBC. Moreover, our study showed that PBC patients in Central Greece are diagnosed at early histological and clinical stages, requiring a high-index of suspicion among healthcare providers, with male patients having a more advanced disease. Last but not least, GLOBE score, a mathematical model to select strategies for treatment and care, applies perfectly in Greek patients. In the near future, hepatologists will be faced with multiple options for PBC therapy and the GLOBE score will likely help to detect patients that may benefit from new therapies.

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Conflict of interests

All authors have declared that no conflict of interest exists.

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