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Correlation between individual autoantibodies and clinical features in primary biliary cholangitis: results of a retrospective longitudinal study

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CORRELATION BETWEEN INDIVIDUAL AUTOANTIBODIES AND CLINICAL FEATURES IN PRIMARY BILIARY CHOLANGITIS: RESULTS OF A RETROSPECTIVE LONGITUDINAL STUDY

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RESUMO

Antecedentes e Objetivos: A colangite biliar primária (CBP) é uma doença hepática imunomediada. O perfil imunológico parece estar relacionado com o prognóstico clínico. No entanto, faltam estudos longitudinais que os correlacionem. Este estudo visa determinar o papel de autoanticorpos específicos no decurso da doença hepática, bem como a sua implicação na resposta ao tratamento com o ácido ursodesoxicólico (AUDC).

Métodos: Foram identificados os doentes que, entre janeiro de 2016 e dezembro de 2020, realizaram estudo imunológico hepático pelo Departamento de Imunologia do Centro Hospitalar Universitário do Porto. Todos os dados clínicos, analíticos e imagiológicos foram obtidos a partir de registos clínicos eletrónicos. Foram utilizados os testes de qui-quadrado, teste exato de Fisher e de Mann-Whitney, na avaliação da relação entre o perfil de autoanticorpos e os parâmetros bioquímicos, evolução clínica e *scores* de resposta à terapêutica com AUDC.

Resultados: Cento e quarenta e três dos 317 doentes foram diagnosticados com CBP (37 dos quais com variante hepatite autoimune), com um seguimento mediano de 6.8 anos. Os anticorpos antimitocondriais foram identificados em 91.6%, os anti-gp210 em 18.2% e os anti-Sp100 em 19.6% dos doentes. A incidência de morte por causa hepática foi mais comum nos doentes com variante de hepatite autoimune concomitante ($p = 0.022$). A presença de cirrose e/ou hipertensão portal, ao diagnóstico ou no final do seguimento, não estava relacionada com a presença de nenhum dos autoanticorpos estudados, nem havia relação com a probabilidade de morte ou de transplante hepático. Os doentes com positividade para anti-Sp100 tinham títulos, à inclusão, de aspartato transglutaminase ($p = 0.007$) e alanina aminotransferase ($p = 0.025$) aumentados. No entanto, os níveis de IgM eram mais baixos neste grupo ($p = 0,005$). Os doentes com anti-gp210 positivo apresentaram uma maior probabilidade de ter uma sobrevida livre de transplante hepático mais baixa e um risco de transplante de fígado ou morte de causa hepática mais elevado [5 ($p = 0.014$), 10 ($p = 0.038$) e 15 anos ($p = 0.041$), usando o *score* de GLOBE, e 5 ($p < 0.001$), 10 ($p = 0.001$) e 15 anos ($p = 0.002$), aplicando o *score* UK-PBC].

Conclusões: Os nossos resultados confirmam uma forte associação entre a positividade de anticorpos anti-gp210 e pior prognóstico, quando aplicados os *scores* de risco UK-PBC e GLOBE. A associação entre a positividade dos anticorpos anti-Sp100 e a lesão hepática, expressa pela elevação das enzimas hepáticas, requer uma maior elucidação e caracterização.

Palavras-chave: Colangite biliar primária, anticorpos antimitocôndria, anti-gp210, anti-Sp100, cirrose, hipertensão portal.

ABSTRACT

Background and Aims: Primary biliary cholangitis (PBC) is an immune mediated liver disease. The allied immunological profile seems to relate to clinical prognosis. Yet, longitudinal studies correlating one to the other are lacking. This study aims to determine the role of a panel of specific autoantibodies in the course of liver disease, as well as its implication on the response to therapy with ursodeoxycholic acid (UDCA).

Methods: Between January 2016 and December 2020, patients who underwent a liver immunological profile analysis were identified by the Immunology Department of Centro Hospitalar Universitário do Porto. All clinical, analytical and imagiological data were extracted from electronic clinical records. Chi-square test or Fisher's exact test and Mann-Whitney test, as appropriate, were used to evaluate the relationship between the presence of autoantibodies and biochemical parameters, clinical outcomes and therapeutic response scores to UDCA.

Results: One hundred and forty three patients out of 317 were diagnosed with PBC (37 with autoimmune hepatitis variant), with a median follow up of 6.8 years. Antimitochondrial antibodies were present in 91.6%, anti-gp210 in 18.2% and anti-Sp100 in 19.6% of the patients. The incidence of liver-related death was more common in patients with concurrent autoimmune hepatitis variant ($p = 0.022$). Cirrhosis and/or portal hypertension at baseline or at the end of follow-up was not linked to the presence of any of the autoantibodies studied. No relationship was also found with the probability of dying or being transplanted. Patients with positivity to anti-Sp100 antibodies were more likely to have increased baseline levels of aspartate aminotransferase ($p = 0.007$) and alanine aminotransferase ($p = 0.025$). Yet, IgM levels were lower in this group ($p = 0.005$). Patients with positive anti-gp210 were more likely to have a lower median transplant free survival rate and higher median risk of liver transplant or liver-related death [5- ($p = 0.014$), 10- ($p = 0.038$) and 15-years ($p = 0.041$) using the GLOBE score, and 5- ($p < 0.001$), 10- ($p = 0.001$) and 15-years ($p = 0.002$), when applying the UK-PBC score].

Conclusions: Our findings confirm a strong association between anti-gp210 antibodies positivity and a worse outcome when applying UK-PBC and GLOBE risk scores. The association between anti-Sp100 positivity and hepatic lesion, as expressed by increased liver enzymes, require further elucidation and characterization.

Keywords: Primary biliary cholangitis, antimitochondrial antibodies, anti-gp210, anti-Sp100, cirrhosis, portal hypertension.

ABBREVIATIONS LIST

AASLD – American Association for the Study of the Liver Diseases

AIH – Autoimmune hepatitis

ALB – Albumin

ALP – Alkaline Phosphatase

ALT – Alanine aminotransferase

AMA – Antimitochondrial antibody

ANA – Antinuclear antibody

Anti-gp210 – Anti-glycoprotein-210 antibody

Anti-PML – Anti-promyelocytic leukemia protein antibody

AST – Aspartate aminotransferase

CT – Computed Tomography

EASL – European Association for the Study of the Liver

FS – Fibroscan®

GGT – Gamma-glutamyltransferase

HBV – Hepatitis B virus

HCC – Hepatocellular Carcinoma

HCV – Hepatitis C virus

HIV – Human Immunodeficiency Virus

IgM – Immunoglobulin M

MRI – Magnetic Resonance Imaging

PBC – Primary Biliary Cholangitis

PLT – Platelet Count

PT – Prothrombin Time

SD – Standard Deviation

SS – Sjögren Syndrome

TBIL – Total Bilirubin

UDCA – Ursodeoxycholic Acid

UDE – Upper Digestive Endoscopy

US - Ultrasound

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INTRODUCTION

Primary biliary cholangitis (PBC), formerly called primary biliary cirrhosis until 2015[1], is an autoimmune liver disorder. Being more frequent among women, it is defined as the presence of 2 out of 3 criteria amongst chronic cholestasis [particularly alkaline phosphatase (ALP) more than 1,5 times the upper limit of normal], presence of antimitochondrial antibodies (AMA) over 1:40 titer, and/or liver biopsy with histological findings consistent with PBC[2,3]. Related risk factors are not yet fully understood, but it is believed that environmental triggers may initiate the immune response in genetically susceptible individuals[4–6]. The clinical course is characterized by progressive chronic destruction of intrahepatic bile ducts with portal inflammation, potentially evolving to a more advanced stage with increased degrees of fibrosis, cirrhosis and associated complications (portal hypertension, liver failure, hepatocellular carcinoma, among others), which can require liver transplantation[7]. Early recognition of this entity and immediate institution of therapy are the main prognostic modifiers[2]. Ursodeoxycholic acid (UDCA) is recommended for all patients with PBC, with administered dose of 13–15 mg/kg per day[2,3]. The biochemical response to UDCA predicts long-term outcome in PBC, which can be assessed using qualitative definitions based on discrete binary variables or quantitative scoring systems computed from continuous parameters[2].

Antimitochondrial antibodies are present in up to 90% of patients with PBC[5]. Yet, other autoantibodies may exist and can be used for diagnosis, particularly the anti-Sp100 and anti-glycoprotein-210 antibodies (anti-gp210), which are specific antinuclear autoantibodies (ANA) directed towards nuclear antigens or envelope proteins, with a specificity greater than 95% for PBC[4,8,9]. Granito *et al* showed that the concomitant positivity for both anti-Sp100 and anti-gp210 antibodies showed a 100% positive predictive value for PBC, irrespective of the AMA status[10]. The presence of these autoantibodies seems to be associated with an unfavorable course of the disease, regardless of the bilirubin value (which has, per se, an impact on prognosis)[7,11–13], as well as the presence of anti-centromere antibodies that have been associated to a phenotype of greater expression of portal hypertension[14–16]. Anti-hexokinase 1 and anti-kelch-like 12 antibodies, besides being very specific and prevalent[17], seem to be related with shorter survival, reduced time to liver decompensation and transplantation[18].

Notwithstanding the apparent relationship between the immunological profile and clinical prognosis, there is still a lack of longitudinal studies correlating one to the other, in order to definitively consider them with prognostic value, an issue that has also been acknowledged by the EASL[2]. Therefore, we performed this retrospective longitudinal study to determine the role of a

panel of autoantibodies in the clinical history of PBC, as well as their implication on the response to therapy with UDCA.

METHODS

Patient selection and study design

This retrospective longitudinal cohort study enrolled patients who underwent a liver immunological profile analysis, for either the diagnosis or follow-up of liver diseases, at the Immunology Department of Centro Hospitalar Universitário do Porto, Portugal, between January 2016 and December 2020.

Briefly, this study included all patients 18 years old or older, with a definitive diagnosis of PBC, based on the presence of at least 2 out of 3 criteria: i) positive AMA; ii) elevated ALP [and/or gamma-glutamyl transferase (GGT)]; iii) liver biopsy concordant with PBC diagnosis. Patients having negative AMA but with positivity for anti-gp210 and/or anti-Sp100 in association with cholestasis (provided that other causes for altered liver profile were excluded) and/or liver biopsy compatible with PBC, were also included. Individuals whose follow-up was carried out at another Institution, with liver failure at enrollment, cirrhosis of other etiologies, including hepatotropic virus infection with hepatitis B (HBV) or hepatitis C virus (HCV), history of alcohol abuse, human immunodeficiency virus (HIV) infection, or patients with insufficient data for clinical evaluation were excluded from the study. Patients with Autoimmune hepatitis (HAI)-PBC variant syndrome, according to the Paris criteria[2,19], or a concordant liver biopsy, were also included and considered for analysis.

The Hospital Board and Ethics Committee of Centro Hospitalar Universitário do Porto approved the study [approval number 2021.244(196-DEFI/204-CE)]. Data was duly anonymized, and the study protocol was conducted according to the ethical principles of the Declaration of Helsinki.

Study variables

Data was collected retrospectively by consulting the electronic medical record of each patient. Demographic characterization, health status, laboratory data and clinical presentation at diagnosis, at the date of the immunological study and after 12 months were collected. This included sex, age, serum biochemical data [alanine aminotransferase (ALT), aspartate aminotransferase (AST), ALP, GGT, total bilirubin (TBIL), and albumin (ALB)], platelet count (PLT), prothrombin time (PT), immunological data (immunoglobulins, ANA by indirect immunofluorescence and autoantibodies associated with hepatic autoimmune disorders), imaging [abdominal ultrasound (US), magnetic resonance imaging (MRI) and/or computed tomography (CT), upper digestive endoscopy (UDE)] and histologic stage, when available. In the case of patients with more than one immunological study, the one with the most analytical parameters evaluated was adopted.

Outcomes

The primary outcomes were liver-related death [death attributable to hepatic decompensation, liver failure or hepatocellular carcinoma (HCC)] or liver transplant. The secondary outcome was liver disease progression, as expressed by the presence of cirrhosis with or without portal hypertension. The degree of fibrosis was categorized in four stages (from F0 – no liver fibrosis, to F4 – liver cirrhosis), based on liver biopsy or elastography result given by Fibroscan® (FS) data, with cut-offs settled for different stages of liver fibrosis as according to Karlas *et al* and Corpechot *et al*[20,21]. Cirrhosis diagnosis was made once the patient had an elastography result compatible with F4, liver dysmorphia in any imaging exam and/or the presence of decompensation resulting of unequivocal clinical expression of portal hypertension (hepatic encephalopathy, ascites, presence of esophageal/gastric varices). Portal hypertension was defined as a composite including the development of one or several of the following features: i) presence of esophageal and/or gastric varices and/or hypertensive gastropathy as evaluated by UDE; ii) presence of ascites; iii) presence of portosystemic shunts evaluated by imaging exams. Our primary goal was to analyze and determine the impact of a specific autoantibody in the risk of death, transplantation and progression of the liver disease towards cirrhosis and portal hypertension.

In order to assess the role of the immunological profile in prediction of response to UDCA treatment, biochemical data was also analyzed after 1 year of treatment. Two scores were used: the UK-PBC Risk Score and the GLOBE score[22,23]. The first one estimates the risk of a liver transplant or liver-related death occurring within in 5, 10, or 15 years[23]. The second one stratifies patients to high or low risk according to a calculated score, and assesses the transplant free survival rates at 5, 10 and 15 years[22]. Qualitatively, the Barcelona, Paris-I, Paris-II, Rotterdam and Momah-Lindor criteria were also used to evaluate the treatment response[24–28].

Statistical analysis

Characteristics of participants are described by absolute and relative frequencies and compared through the Chi-square test or Fisher's exact test, as appropriate, in categorical variables. Medians and standard deviations (SD) were used to describe continuous variables compared using the Mann-Whitney test. Data analysis was performed using the SPSS Statistic 26.0 (IBM SPSS, New York, USA). A significance level of 0.05 was used.

RESULTS

Characterization of the studied population

During the 5-year study period, liver immunological profile was analyzed in 317 patients, 149 of which had a final diagnosis of PBC. Of these, 6 were excluded due to HBV and/or HCV concomitant infection. One hundred and forty-three patients were considered for final analysis (37 with AIH variant and 106 with "pure" PBC) (Fig. 1). The baseline characteristics are presented in Table I. Median follow up was of 6.8 years for all the patients, with AIH variant patients with a longer period of surveillance of 8.6 years. Patients were more commonly female (88.1%), with PBC diagnosis established at the age of 54.5 years old. When evaluated, AMA were present in 91.6%, anti-gp210 in 18.2%, anti-Sp100 in 19.6% and anti-centromere in 5.6% of patients. Cirrhosis was already documented at diagnosis in 1/4 and portal hypertension in 1/10 of patients. Patients with higher AST ($p = 0.002$), ALT ($p < 0.001$) and TBIL ($p < 0.001$), as well as those with AMA titers ≥ 1280 ($p = 0.028$) were significantly more likely to have AIH variant.

At diagnosis, half of the patients were asymptomatic. Among those with symptoms, pruritus was the most frequent complaint in 24.5%, followed by asthenia in 21% of the patients. The incidence of abdominal pain was significantly more common in the AIH-PBC variant group than in the PBC group (11.4% vs. 1%; $p = 0.016$) (Table II). Concomitant autoimmune diseases were found in 28.7% of the patients, being Sjögren Syndrome (SS) the most common (10.2%), and more likely to be diagnosed in the PBC-AIH group (Table III).

Prognostic value of autoantibodies

Patients with positivity to anti-Sp100 antibodies were more likely to have increased baseline levels of AST ($p = 0.007$) and ALT ($p = 0.025$). Yet, IgM levels were lower in this group ($p = 0.005$). Portal hypertension and/or cirrhosis at baseline or at the end of follow-up was not related to the presence of any of the autoantibodies studied (AMA, anti-Sp100 and anti-gp210 autoantibodies), as there was no relationship with the probability of dying or being transplanted (Table IV).

Ursodeoxycholic acid biochemical response was assessed by qualitative and quantitative means. Considering the first one, there seems to be a tendency to absence of response when using the Barcelona score for those patients with positive anti-gp210 autoantibodies ($p = 0.068$), although not statistically significant. Using quantitative measures of response, patients with positive anti-gp210 antibodies were likely to have a lower median transplant-free survival rate, when computing probabilities at 5- ($p = 0.014$), 10- ($p = 0.038$) and 15-years ($p = 0.041$) using the GLOBE score, and also an increased median risk of a liver transplant or liver-related death occurring at 5- ($p < 0.001$),

10- ($p = 0.001$) and 15-years ($p = 0.002$), when applying the UK-PBC score. No statistically significant differences were found in other groups ($p > 0.05$) (Table IV).

The incidence of liver-related death was more common in the AIH-PBC variant group than in the PBC group (13.5% vs. 3.8%, $p = 0.022$).

DISCUSSION

Thus far, few studies focus on the role of immunological liver profile on liver disease progression, rate of progression, related survival and time free of liver transplantation. This issue is even more difficult to address as the institution of therapies (UDCA) that are known to positively modify the natural course of the disease alters the natural history of liver disease progression in PBC patients.

Our longitudinal retrospective study goes in line with the current notion that PBC is more frequently diagnosed in middle-aged females[29], with an almost 9:1 female:male ratio, and with AMA positivity in up to 90% of the patients[13]. Also, our epidemiological results do not differ from those recently published by Helena Cortez-Pinto *et al* in the largest Portuguese nationwide PBC published study, probably reflecting the same genetic and environmental background.[30]

Similarly to other studies, more than half of the patients were asymptomatic at diagnosis and pruritus and fatigue were the most frequent complaints of those with symptoms[30,31]. The high proportion of asymptomatic patients may be related to i) the particular interaction physician-patient and to the ease with which patient spontaneously complains; ii) the questionnaire conducted by the doctor that may depend on the degree of training and different clinical speciality; and iii) the increased number of patients that are diagnosed of PBC after the investigation of an abnormal liver biochemical profile in an otherwise asymptomatic patient. Prince *et al* found that asymptomatic PBC can be regarded as a temporary phenomenon, as in this study, only 17.4% of surviving patients were symptom free 10 years from the date of diagnosis[31].

Patients with PBC have up to a 60% chance to have an association with at least another autoimmune condition[32]. We found concomitant autoimmune diseases in 28.7% of patients. Among the rheumatologic diseases, SS is the most common condition, occurring in proportion ranging from 3.5 to nearly 100% of cases[32], which was also verified in our study. A recent study presented that although autoimmune diseases are prevalent in the PBC population the co-existence does not seem to influence the clinical outcomes of PBC[33].

The AIH variant was present in one quarter of PBC patients, which is a number close to the reported PBC cohorts with this variants (3-19%)[34,35]. This group was more likely to have increased baseline levels of AST, ALT, TBIL and AMA. These findings may be related to a more important liver injury associated with flares of hepatitis. Additionally, the incidence of liver-related death was more common in the AIH-PBC variant group. The reasons for the poorer survival in the patient with AIH features are not clear but could be influenced by disease severity. Yang *et al.* demonstrated that patients with AIH features had higher transaminases and TBIL at diagnosis and a diminished 5-year

adverse-outcome-free survival than patients with classical PBC[36]. These results are also in accordance with Silveira *et al*[37]. Still, there is a lack of studies on AIH-PBC variant, both in relation to its clinical presentation and its natural history. These studies are important, as the prevalence of the variant is significant and may have an influence on treatment options and clinical management of patients.

Thus far, the role of the immunological profile in PBC patients and outcome has not been extensively studied, with the reported series commonly small-sized. To the best of our knowledge, only eight studies have addressed this issue, being the majority of them in populations in Asia and with a retrospective character[12–14,38–42]. The proportion of patients with positive anti-gp210 and anti-Sp100 antibodies in our study is similar to that found in other studies (approximately 20%)[9,14,42,43], even though probably underestimated, as not all patients underwent the complete immunological liver profile. No statistical significance was found with the outcomes of death, liver transplant, cirrhosis or portal hypertension and the autoantibodies, which may possibly be justified by the short follow-up time and/or the lack of imaging data to support the diagnosis of cirrhosis with or without portal hypertension, since some patients, in the absence of specific complaints and cirrhosis at inclusion, were followed only with biochemical data, without repetition of liver imagery or elastography, irrespectively of current recommendations.

Patients with positive anti-Sp100 were more likely to have increased baseline levels of AST and ALT, which may reflect greater hepatic injury in these patients. There is lack of recent studies regarding the role of anti-Sp100 in the disease outcome; nevertheless, it has been proposed that anti-promyelocytic leukemia protein (anti-PML) antibodies and anti-Sp100 double reactivity identifies a subgroup of patients with more advanced liver disease compared to those who lack reactivity to these antigens and is associated with disease severity and poor prognosis[44]. Unfortunately, the anti-PML antibody has not been investigated in the population, as it was not requested in the routine patient study. Our results warrant further investigation on the role of anti-Sp100 on liver inflammation.

Patients with positive anti-gp210 antibodies presented a tendency to absence of response to therapy when using the Barcelona score. This criterium assesses response to therapy only by considering the decrease in ALP[24], which may indicate that patients with anti-gp210 maintain high ALP levels despite UDCA treatment, so an eventual propensity for a need of a second line therapy may be necessary. Through the GLOBE and UK-PBC scores, patients with positive anti-gp210 were likely to have a lower median transplant-free survival rate and higher median risk of a liver transplant or liver-related death. Even though we found this important association, unfortunately

and probably due to the short-term follow up period, we were not able to verify these same corresponding results when considering the outcomes of the patients. This is consistent with other studies who have checked that, as a biomarker, anti-gp210 antibody was associated with a more severe cholestatic manifestation and a worse long-term prognosis[40,41]. Furthermore, in a recent study, positivity for anti-gp210 antibody was independently associated with a higher risk of liver-related death or transplantation[13]. Nakamura *et al* hypothesized that the presence of anti-gp210 is associated with liver failure type progression[16,43] and that it's serial quantitation is useful for monitoring the effect of UDCA and for the early identification of patients at high risk for end-stage hepatic failure[39].

The limitations of this study are its retrospective character and limited follow-up period resulting in a decreased power when considering the analyzed outcomes (survival, time free of liver transplant). Also, and due to its design, there were still many patients who did not have evaluation of all the immunological liver profile, underestimating their prevalence and impact on the outcomes. Yet, and even though being a single-center study, it gathered an important number of patients with a rare disease as is PBC, as Centro Hospitalar Universitário do Porto is a tertiary reference Institution for the follow-up of liver disease patients. This is also the first Portuguese study addressing specifically this issue.

CONCLUSION

In conclusion, this longitudinal retrospective study points to the importance of addressing anti-gp210 to establish prognosis, proposing that, in future prognostic models this item might be considered for inclusion in prognostic scores. The mechanisms how anti-Sp100 leads to increased liver enzymes must be addressed in future works if one wants to optimise targeting this autoantibody in the management of PBC.

Conflict of interest declaration: All the authors have nothing to disclose

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APPENDIX

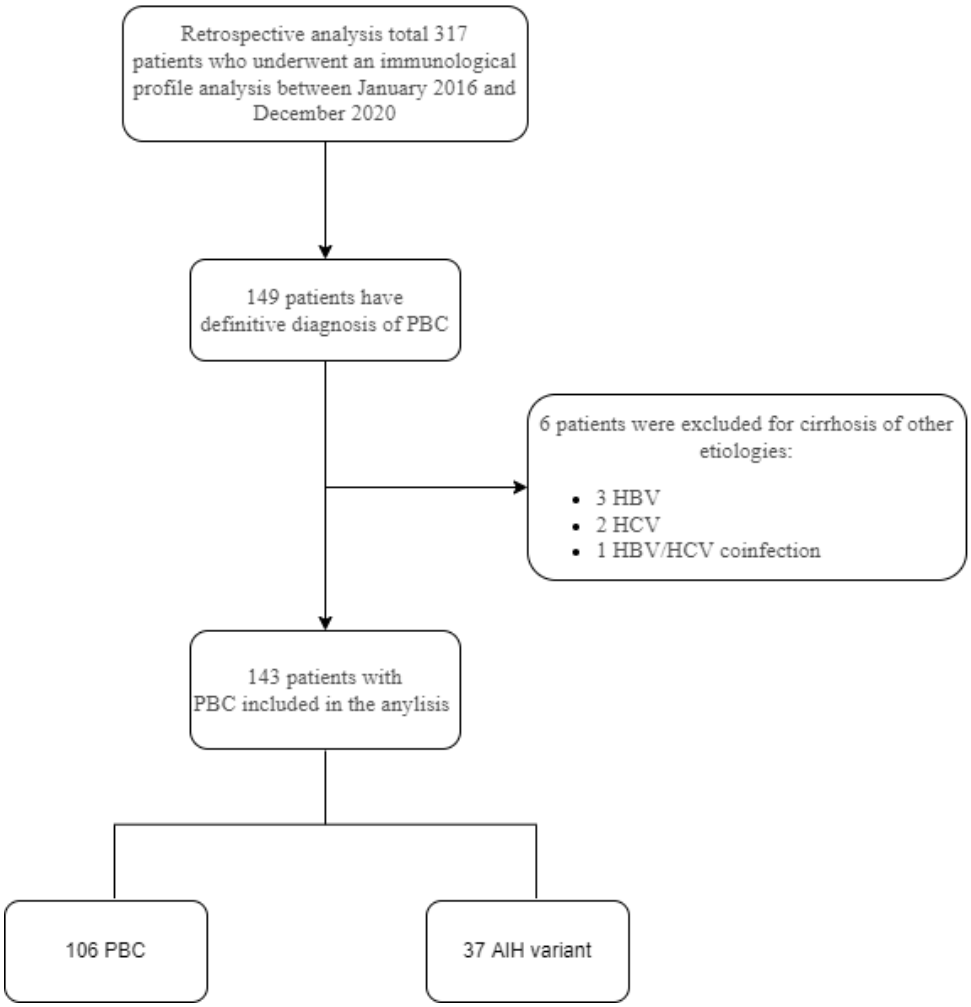

Figure 1. Flow diagram showing derivation of the studied cohort.

Abbreviations: PBC, primary biliary cholangitis; HBV, hepatitis B virus; HCV, hepatitis C virus; AIH, Autoimmune hepatitis.

Table I. Demographic, clinical and laboratory findings in patients with PBC and with AIH variant at inclusion.

Variables	**Total (n = 143)**	**PBC (n = 106)**	**PBC + AIH (n = 37)**	**p-value**
Age (years)	54.5 (13.3)	55.7 (13.3)	51.1 (12.8)	0.762
Female, n (%)	126 (88.1)	93 (87.7)	33 (89.2)	0.814
AMA+	131 (91.6)	95 (89.6)	36 (97.3)	0.147
AMA ≥ 1280	73 (51.0)	48 (58.5)	25 (80.6)	**0.028**
AMA < 1280	40 (28.0)	34 (41.5)	6 (19.4)	
Anti-gp210+	26 (18.2)	20 (29.9)	6 (26.1)	0.731
Anti-Sp100+	28 (19.6)	20 (31.7)	8 (32.0)	0.982
Anti-centromere	8 (5.6)	5 (4.7)	3 (8.1)	1.000*
AST (mean; SD)	75.5 (231.0)	49.4 (36.2)	155.4 (457.8)	**0.002**
ALT (mean; SD)	78.9 (129.7)	59.8 (42.9)	137.2 (244.3)	**<0.001**
ALP (mean; SD)	242.3 (218.1)	239.0 (231.3)	252.8 (173.5)	0.629
GGT (mean; SD)	235.2 (231.3)	236.5 (245.6)	231.0 (181.9)	0.533
TBIL (mean; SD)	1.18 (3.2)	0.7 (1.31)	2.5 (6.1)	**<0.001**
IgM (mean; SD)	283.5 (173.3)	277.7 (178.4)	300.4 (160.0)	0.725
Portal Hypertension at time of diagnosis	16 (11.2)	11 (28.2)	5 (33.3)	0.712
Cirrhosis at time of diagnosis	32 (22.4)	20 (20.6)	12 (33.3)	0.127
Death	9 (6.3)	4 (3.8)	5 (13.5)	**0.036**

*Fisher; bold values correspond to p-value < 0.05

Abbreviations: PBC, primary biliary cholangitis; AIH, Autoimmune hepatitis; AMA, antimitochondrial antibody; Anti-gp210, anti-glycoprotein-210 antibody; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; TBIL, total bilirubin; IgM, Immunoglobulin M.

Table II. Clinical presentation/symptoms commonly found in patients by the time of diagnosis

Variables	Total (n = 143)	PBC (n = 106)	PBC + AIH (n = 37)	p-value
Asymptomatic	76 (53.1)	61 (57.5)	15 (40.5)	0.074
Symptomatic	67 (46.9)	45 (42.5)	22 (59.5)	
Asthenia	30 (21.0)	21 (19.8)	9 (24.3)	0.527
Pruritus	35 (24.5)	23 (21.7)	12 (32.4)	0.180
Abdominal pain	5 (3.5)	1 (1.0)	4 (11.4)	**0.016***
Anorexia	3 (2.1)	2 (1.9)	1 (2.7)	1.000*
Nausea/vomiting	7 (4.9)	5 (4.7)	2 (5.4)	1.000*
Jaundice	7 (4.9)	3 (2.8)	4 (10.8)	0.078*
Oedema	4 (2.8)	2 (1.9)	2 (5.4)	0.285*
Ascites	7 (4.9)	3 (2.8)	4 (11.1)	0.079*
Encephalopathy	2 (1.4)	0 (0)	2 (5.4)	0.063*
Weight loss	7 (4.9)	5 (4.7)	2 (5.4)	1.000*
Haematemesis/melena	1 (0.7)	1 (0.9)	0 (0)	1.000*
Xerostomia	12 (8.4)	10 (9.4)	2 (5.4)	0.731*
Xerophthalmia	12 (8.4)	9 (8.5)	3 (8.1)	1.000*

*Fisher; bold values correspond to p-value < 0.05

Abbreviations: PBC, primary biliary cholangitis; AIH, Autoimmune hepatitis.

Table III.
Systemic autoimmune diseases identified by the time of diagnosis of primary biliary cholangitis (PBC) or with its autoimmune hepatitis (AIH) variant.

Autoimmune diseases	**Total (n = 143)**	**CBP (n = 106)**	**CBP + HAI (n = 37)**	**p-value**
Sjögren	15 (10.5)	6 (6.0)	9 (25.0)	**0.004**
Raynaud	6 (4.2)	4 (4.0)	2 (5.6)	0.655*
Scleroderma	10 (7.0)	6 (6.1)	4 (11.1)	0.322
Autoimmune thyroiditis	6 (4.2)	3 (3.0)	3 (3.0)	0.192*
SLE	2 (1.4)	2 (2.0)	0 (0)	1.000*
Celiac disease	2 (1.4)	2 (2.0)	0 (0)	1.000*

*Fisher; bold values correspond to p-value < 0.05
Abbreviations: PBC, primary biliary cholangitis; AIH, Autoimmune hepatitis; SLE, Systemic lupus erythematosus.

Table IV.
Association between clinical features, UDCA biochemical response and autoantibodies of study participants

Variables	AMA+	AMA-	p-value	Anti-gp210+	Anti-gp210-	p-value	Anti-Sp100+	Anti-Sp100-	p-value
Age (years)	54.6 (13.4)	52.8 (12.2)	0.652	54.4 (15.2)	53.3 (12.3)	0.501	51.0 (12.6)	53.8 (12.6)	0.867
Female	115 (87.8)	11 (91.7)	1.000*	21 (80.8)	58 (90.6)	0.285*	23 (82.1)	56 (93.3)	0.136*
AST (mean; SD)	78.9 (242.5)	42.7 (28.4)	0.539	57.4 (34.0)	99.1 (346.9)	0.291	162.7 (534.0)	56.8 (41.7)	**0.007**
ALT (mean; SD)	81.6 (135.8)	53.0 (29.5)	0.392	72.8 (56.6)	89.8 (185.4)	0.468	128.1 (26.0)	68.1 (62.3)	**0.025**
ALP (mean; SD)	240.4 (257.2)	260.4 (257.2)	0.338	311.0 (339.8)	241.6 (208.2)	0.282	268.8 (238.8)	263.7 (263.6)	0.928
GGT (mean; SD)	234.5 (215.2)	241.8 (366.0)	0.393	298.2 (202.1)	298.2 (202.1)	0.270	294.9 (311.7)	262.5 (246.2)	0.171
TBIL (mean; SD)	1.2 (3.4)	0.3 (0.1)	0.223	0.62 (0.37)	1.5 (4.6)	0.098	1.86 (6.2)	1.0 (2.3)	0.075
IgM (mean; SD)	289.0 (177.2)	229.3 (124.3)	0.155	365.3 (224.4)	271.8 (178.1)	0.401	249.0 (107.7)	318.5 (219.5)	**0.005**
IgG (mean; SD)	1440.1 (631.0)	1357.2 (596.4)	0.932	1357.3 (604.2)	1508.4 (466.1)	0.490	1568.6 (588.1)	1458.1 (802.6)	0.388
Portal Hypertension at time of diagnosis	16 (33.3)	6 (100)	0.163*	2 (15.4)	8 (42.1)	0.141*	2 (22.2)	7 (35)	0.675*
Cirrhosis at time of diagnosis	32 (26.2)	0 (0)	0.065*	5 (20.8)	14 (22.6)	0.861	4 (15.4)	13 (22.4)	0.459
Portal Hypertension	33 (67.3)	0 (0)	0.120*	5 (83.3)	12 (63.2)	0.624*	5 (71.4)	11 (73.3)	1.000*
Cirrhosis	50 (44.6)	0 (0)	0.070	5 (45.5)	11 (42.3)	1.000*	8 (47.1)	18 (34.0)	0.331
Liver Transplant	7 (5.3)	0 (0)	1.000*	2 (7.7)	2 (3.1)	0.576*	1 (3.6)	3 (5.0)	1.000*
Death	9 (6.9)	0 (0)	1.000*	3 (11.5)	2 (3.1)	0.143*	0 (0)	5 (8.3)	0.173*
UDCA biochemical response									
Barcelona	64 (62.1)	5 (50.0)	0.508*	8 (44.4)	37 (68.5)	0.068	13 (61.9)	30 (63.8)	0.879
Paris I	83 (80.6)	9 (90.0)	0.685*	13 (72.2)	46 (85.2)	0.289*	18 (85.7)	38 (80.9)	0.742*
Paris II	64 (62.1)	7 (70.0)	0.742	10 (55.6)	36 (66.7)	0.395	14 (66.7)	29 (61.7)	0.695
Rotterdam	80 (84.2)	10 (100)	0.351*	13 (81.3)	46 (90.2)	0.385*	20 (95.2)	36 (85.7)	0.408*
Momah-Lindor	91 (88.3)	10 (100)	0.596*	15 (83.3)	48 (88.9)	0.682*	19 (90.5)	41 (87.2)	1.000*
GLOBE score ≤ 0.3	67 (69.8)	7 (87.5)	0.433*	15 (78.9)	23 (63.6)	0.231	17 (85.0)	26 (61.9)	0.065
GLOBE score > 0.3	29 (30.2)	1 (12.5)		4 (21.1)	16 (36.4)		3 (15.0)	16 (38.1)	

GLOBE 5 years	91.5 (12.8)	95.4 (4.7)	0.348	87.5 (21.3)	93.9 (7.5)	**0.014**	95.3 (3.5)	91.5 (14.8)	0.105
GLOBE 10 years	81.9 (19.3)	88.6 (11.2)	0.402	77.0 (24.9)	85.5 (14.6)	**0.038**	88.2 (8.6)	82.5 (19.9)	0.100
GLOBE 15 years	72.9 (22.6)	81.3 (17.1)	0.533	67.4 (27.4)	77.2 (19.0)	**0.041**	80.4 (13.6)	74.0 (23.0)	0.149
UK-PBC 5 years	2.8 (7.9)	0.7 (0.4)	0.260	2.7 (4.5)	1.5 (1.5)	**<0.001**	1.2 (1.3)	2.0 (3.1)	0.065
UK-PBC 10 years	7.3 (13.2)	2.6 (1.5)	0.123	8.2 (12.7)	4.9 (4.9)	**0.001**	4.1 (4.2)	6.4 (8.8)	0.066
UK-PBC 15 years	11.8 (16.4)	4.8 (2.8)	0.072	13.5 (19.1)	8.8 (8.4)	**0.002**	7.4 (7.3)	11.0 (13.7)	0.071

*Fisher; bold values correspond to p-value < 0.05

Abbreviations: AMA, antimitochondrial antibody; Anti-gp210, anti-glycoprotein-210 antibody; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; TBIL, total bilirubin; IgM, Immunoglobulin M; IgG, Immunoglobulin G; UDCA, ursodeoxycholic acid.

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