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Anti-gp210 and anti-Sp100 antibodies in primary biliary cholangitis

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ABSTRACT

Background and study aims: To determine the sensitivity and specificity of anti-gp210 and anti-Sp100 autoantibodies in primary biliary cholangitis (PBC)

Patients and Methods: Sera of 106 PBC patients with positive anti-mitochondrial antibodies and 58 healthy blood donors were analyzed. A line immunoassay was used to evaluate the reactivity of anti-gp210 and anti-Sp100 antibodies.

Results: The frequency of anti-gp210 and anti-Sp100 autoantibodies was 29.2% and 28.3%, respectively. Eight patients had both anti-gp210 and anti-Sp100 antibodies. Of 106 patients, 23 (21.7%) had anti-gp210 antibody, although not anti-Sp100 antibody, and 22 (20.7%) had anti-Sp100, although not anti-gp210 antibodies. Their combination increased the frequency of anti-gp210 and anti-Sp100 antibodies from 29.2% to 50% ($P = 0.002$) and 28.3% to 50% ($P = 0.0012$), respectively. In the control group, two subjects had anti-gp210 antibody and none had anti-Sp100 antibody. Thus, the specificity of anti-gp210 and anti-Sp100 antibodies was 96.5% and 100%, respectively. The positive predictive value (PPV) of anti-gp210 antibody was 94%; its negative predictive value (NPV) was 42.7%. The PPV and NPV of anti-Sp100 antibody were 100% and 43.3%, respectively.

Conclusion: It is important to combine anti-gp210 and anti-Sp100 antibodies in the immunological exploration of PBC.

Introduction

Primary biliary cholangitis (PBC) is an autoimmune disease of the liver. It is characterized by the chronic inflammatory destruction of intrahepatic bile ducts leading to cholestasis and cirrhosis [1]. M2 antimitochondrial antibodies (AMA) are the serological hallmark of PBC and are found in 90%–98% of PBC patients. PBC-specific antinuclear antibodies, particularly anti-gp210 and anti-Sp100, are used for the diagnosis of PBC in AMA-negative PBC patients [2] and are included in the diagnosis criteria of PBC [3]. Moreover, anti-gp210 antibody has been associated with disease severity [4].

gp210 is an integral membrane protein that anchors the nuclear pore complex to the nuclear membrane [5]. It has a cytoplasmic carboxy-terminal domain, an amino-terminal domain located in the perinuclear space, and a transmembrane segment. The C-terminal tail domain comprises 58 amino acids. In PBC, the anti-gp210 antibody recognizes an epitope, which is contained within a linear stretch of 15 amino acids within the C-terminal domain of the protein.

Sp100 is a nuclear protein of 480 amino acids with an aberrant electrophoretic mobility to 100 kDa. One or more major autoreactive epitopes are localized in the carboxy-terminal half of Sp100. Anti-Sp100 antibody from PBC patients recognize discontinuous or conformation-dependent epitopes [6].

It has been hypothesized that the frequency of PBC-specific antinuclear antibodies (ANA) varies according to the ethnic origin of PBC patients. Furthermore, a significant genetic predisposition

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to have anti-Sp100, but not anti-gp210 antibodies, has been demonstrated [7].

This study aims to determine the frequency of anti-gp210 and anti-Sp100 antibodies, via line immunoassay (LIA), in a cohort of PBC Tunisian patients and assess their correlation with the disease severity given that no study has been performed in Tunisia regarding ANA in PBC.

Patients and methods

Patients

Our retrospective multicenter study included 106 AMA-positive PBC patients. All sera were received from three hospitals in central Tunisia between 2018 and 2019 at the time of PBC diagnosis. All specimens were stored at −80 °C until use. In all patients, PBC diagnosis was based on liver histology and/or liver biochemistry and AMA positivity [8]. The control group comprised 58 healthy blood donors, matched in sex and age with patients, and with no personal or family history of autoimmune diseases. The control sera were collected at the blood transfusion center of the Farhat Hached Hospital in Sousse.

The PBC patients and healthy donors were recruited with the approval of the local ethics committee. Informed consent was obtained from all subjects involved in this study.

Methods

Indirect immunofluorescence

AMAs were detected via indirect immunofluorescence (IIF) using liver, kidney, and stomach rat sections (frozen sections made in our laboratory) as previously described [9].

Lia

As described in our previous study [10], we determined anti-gp210 and anti-Sp100 antibodies using the multiplexed Euroline profile autoimmune liver diseases kit (EUROIMMUN AG, Lübeck, Germany) according to the manufacturer's instructions. The following PBC-associated antigens are contained in this kit: AMA-M2, which is natively purified from bovine heart containing the E2 subunit of the pyruvate dehydrogenase complex; M2-E3, a recombinant fusion protein, including the immunogenetic domains of the E2 subunits of the branched-chain 2-oxoacid dehydrogenase and pyruvate dehydrogenase enriched with 2-oxoglutarate dehydrogenase; sp100 (nuclear granular protein); promyelocytic leukemia protein; and gp210 recombinant proteins (integral protein of the nuclear membrane).

Statistical analysis

All statistical analyses were completed using Epi Info 3.5. The comparison of frequencies of autoantibodies was conducted using the chi-square or Fisher exact test. A *P* value is two-tailed; a *P* value of <0.05 was considered statistically significant.

Results

Demographic characteristics of patients and controls

Table 1 presents the epidemiologic features of patients and control subjects.

Frequency of anti-gp210 and anti-Sp100 antibodies

Table 2 presents the frequency of anti-gp210 and anti-Sp100 antibodies in PBC patients and control group. Anti-gp210 and anti-Sp100 autoantibodies were detected in 31 (29.2%) and 30 (28.3%) patients, respectively. Eight patients had anti-gp210 and anti-Sp100. Of 106 patients, 23 (21.7%) had anti-gp210, although not anti-Sp100, and 22 (20.7%) had anti-Sp100, although not anti-gp210. Their combination increased the frequency of anti-gp210 and anti-Sp100 from 29.2% to 50% (*P* = 0.002) and 28.3% to 50% (*P* = 0.0012), respectively.

Table 1
Epidemiologic features of the PBC patients and control group.

Feature	PBC patients (n = 106)	Control group (n = 58)	*P* value
Sex ratio (F/M)	7.1 (93/13)	6.2 (50/8)	0.78
Mean age	56.4 ± 12.5 years	55 ± 13 years	0.50
Age range	30–84 years	30–78 years	–

Table 2
Frequency of anti-gp210 and anti-Sp100 antibodies in the PBC patients and control group.

Antibody	PBC patients (n = 106)	Control subjects (n = 58)
Anti-gp210	29.2%[a] (31/106)	3.4% (2/58)
Anti-Sp100	28.3%[b] (30/106)	0%
Anti-gp210 or anti-Sp100	50% [a,b] (53/106)	3.4% (2/58)
Anti-gp210 and anti-Sp100	7.5% (8/106)	0%
Anti-gp210 + anti-sp100 -	21.7% (23/106)	3.4% (2/58)
Anti-Sp100 + anti-gp210 -	20.7% (22/106)	0%

a: p = 0.002.
b: p = 0.0012.

Sensitivity, specificity, and positive and negative predictive values of anti-gp210 and anti-Sp100 antibodies

The sensitivity of anti-gp210 and anti-Sp100 antibodies was 29.2% (31/106) and 28.3% (30/106), respectively. Of the 58 subjects from the control group, none had anti-Sp100 and two had anti-gp210; thus, the specificity of anti-gp210 and anti-Sp100 antibodies was 96.5% and 100%, respectively. The positive predictive value (PPV) and the negative predictive value (NPV) of anti-gp210 were 94% and 42.7%, respectively. Additionally, the PPV and NPV of anti-Sp100 were 100% and 43.3%, respectively (Table 3).

Frequency of anti-gp210 and anti-Sp100 according to sex

Table 4 presents the profiles of PBC-specific autoantibodies according to sex. The frequency of anti-gp210 antibody in female

Table 3
Sensitivity, specificity, PPV, and NPV of anti-gp210 and anti-Sp100 antibodies.

	Anti-gp210	Anti-Sp100	Combination Anti-gp210 and Anti-Sp100
Sensitivity	29.2% ± 8.6% (31/106)	28.3% ± 8.6% (30/106)	50% ± 9.5% (53/106)
Specificity	96.5% ± 4.7% (56/58)	100% ± 0% (58/58)	100% ± 0% (58/58)
PPV	94% (31/33)	100% (30/30)	–
NPV	42.7% (56/131)	43.3% (58/134)	–

PPV: positive predictive value, NPV: negative predictive value.

Table 4
Frequency of autoantibodies according to sex.

Antibody	Female (n = 93)	Male (n = 13)
Anti-gp210	30.1%[a] (28/93)	23% (3/13)
Anti-Sp100	25.8%[b] (24/93)	46.1% (6/13)
Anti-gp210 or anti-Sp100	49.4%[a,b] (46/93)	53.8% (7/13)
Anti-gp210 and anti-Sp100	6.4% (6/93)	15.4% (2/13)

a: p = 0.007.
b: p = 0.0008.

and male patients was 30.1% and 23%, respectively; however, the difference was not statistically significant. Male patients had a higher frequency of anti-Sp100 antibody than female patients; however, no statistical difference was observed (46.1% and 25.8%, respectively).

Discussion

IIF on the rodent liver–kidney–stomach remained the most used method for the detection of AMA. Anti-gp210 and anti-Sp100 antibodies are determined using IIF, enzyme-linked immunosorbent assay (ELISA), or LIA.

Using IIF on liver sections or on HEp-2 substrates [11], anti-gp210 and anti-Sp100 antibodies provide rim-like and multiple nuclear dots patterns, respectively. However, IIF could be underestimated. In fact, the immunofluorescence patterns of anti-gp210 and anti-Sp100 antibodies could be masked respectively by concomitant AMA or other ANA.

The sensitivity of ELISA for the detection of anti-gp210 and anti-Sp100 antibodies varied between 17.9% and 51.8%, and 4% and 44%, respectively [12–15] (Table 5). In the present study, LIA was used and found a sensitivity of 29.2% and 28.3% for the anti-gp210 and anti-Sp100 antibodies, respectively. The difference in sensitivity and specificity using ELISA and LIA could be due to the nature of the antigen used in both techniques. Indeed, ELISA has been established using a 15-amino acid epitope on gp210 within the carboxy-terminal end of gp210 and a recombinant Sp100 as antigens. However, in LIA, gp210 recombinant proteins (integral protein of the nuclear membrane) and sp100 (nuclear granular protein) were used.

LIA has been used only in few studies [15–19] (Table 5). Although we and others have used similar LIA from EUROIMMUN, the frequency of anti-gp210 and anti-Sp100 antibodies varied between 18.9% and 40%, and 13.8% and 34.5%, respectively. The frequency of anti-gp210 antibody in the PBC patients in our study was similar to that in the study of Stinton et al., in Canada, [16] and Han et al., in Korea, [15] (29.2%, 27%, and 27.8%, respectively). However, Saito et al., in Japan, [18] found a higher frequency of anti-gp210 in comparison with our study (40% vs. 29.2%). This difference could be because the authors included 12 AMA-negative PBC patients and found that 33.3% of them have anti-gp210, whereas we included only AMA-positive PBC patients in our study. In fact, in the center of Tunisia, no AMA-negative PBC patients have been noted. Indeed, in our laboratory, the sensitivity of IIF for AMA is 98% [9]. Our frequency of anti-Sp100 antibody was similar to that in the study of Stinton et al. [16] (28.3% and 27%, respectively). However, our study showed a higher frequency of anti-Sp100 antibody than that of Saito et al. [18] (28.3% and 13.8%, respectively), although Saito et al. included AMA-negative patients and had the highest frequency of anti-gp210 antibody using LIA. Similarly, in Greece, Gatselis et al. [14], who found the highest frequency of anti-gp210 antibody (51.8%), had the lowest frequency of anti-Sp100 antibody (4.5%) both determined via ELISA. Thus, it can neither be assumed that the frequency varied between studies only because, in some series, AMA-negative PBC patients were also included nor be assumed that the frequency varies according to the assay used. In fact, in the same country, the frequency did not vary according to the assay. For example, in Italy, Muratori et al. [12] and Villalta et al. [19] have used two different assays (ELISA and LIA) and found similar results for anti-gp210 antibody (21% and 18.9%, respectively) and anti-Sp100 antibody (33% and 34.5%, respectively). Moreover, in Korea, Han et al. [15] have used two assays (ELISA and LIA) and found similar frequency for anti-gp210 antibody (22.8% and 27.8%, respectively) and anti-Sp100 antibody (15.2% and 13.9%, respectively). A recent meta-analysis demonstrated that anti-gp210 and anti-Sp100 antibodies had high specificity and low sensitivity for the diagnosis of AMA-negative PBC [2].

Taken together, the discrepancies between different studies may indicate that ethnic/geographical variations could be implicated. The frequency of anti-gp210 antibody in our study (29.2%) is consistent with those found in several countries and equal to the mean frequency of that in previous studies (30%) [3]. Indeed, a mosaic genetic structure of the Tunisian population has been demonstrated [20]. The assignment of Tunisians to multiple ancestries is due to a rich human settlement history.

Several studies have been performed on the frequency of anti-gp210 antibody and its association with PBC activity and severity [21,22]; however, it remains unclear if immune response to gp210 is primarily involved in the pathogenesis of PBC or is subsequent to liver cell destruction. Several questions arise regarding anti-gp210 and PBC.

1- Are anti-gp210 antibodies causes or consequences of PBC?

Gut dysbiosis is another tool added to the puzzle of autoimmunity [23]. The gut microbiota is altered in PBC, and anti–gp210-positive PBC patients had reduced microbiome richness compared with anti–gp210-negative PBC patients [24]. A loss of gut barrier function [25] secondary to gut dysbiosis [23] exposes the liver to an increasing number of intestinal components. Among these are yeast and particularly *Saccharomyces cerevisiae* (*S. cerevisiae*),

Table 5
Frequency of anti-gp210 and anti-Sp100 antibodies in the literature.

Authors (reference)	Year	Country	Assay	Frequency of anti-gp210 (%)	Frequency of anti-Sp100 (%)
Muratori et al. [12]	2008	Italy	ELISA	21	33
Liu et al. [13]	2010	Europe – Japan – USA	ELISA	17.9	18.6
Gatselis et al. [14]	2013	Greece	ELISA	51.8	4.5
Han et al. [15]	2017	Korea	ELISA	22.8	15.2
			LIA	27.8	13.9
Stinton et al. [16]	2010	Canada	LIA	27	27
Mytilinaiou et al. [17]	2012	UK	LIA	–	20.6
Saito et al. [18]	2012	Japan	LIA	40	13.8
Villalta et al. [19]	2015	Italy	LIA	18.9	34.5
Our series	2020	Tunisia	LIA	29.2	28.3

which is one of the most frequent yeasts of the mycobiota [26]. *S. cerevisiae* can translocate from the gut to the liver via the enterohepatic circulation owing to a leaky gut [27]. *S. cerevisiae* is known to be a trigger for several autoimmune diseases, including PBC [28]. Interestingly, the gp210 of *S. cerevisiae* is similar to the human gp210 [29]. Molecular mimicry is among the mechanisms of the breakdown of immunological tolerance against self-antigens. Hence, the presence of gp210 on *S. cerevisiae* could be responsible for cross-recognition by immune cells leading to epitope spreading among self-antigens. Thus, anti-human gp210 antibodies are produced and subsequently bind to the liver gp210. Immune complexes anti-gp210 antibodies-gp210 induce complement activation, which has been demonstrated in PBC [30]. Thereafter, intrahepatic biliary epithelial cell (BEC) destruction will ensue. Since gp210 is highly expressed on BEC [31], *thus, could we imagine that BEC expresses gp210 on apotopes (apoptotic blebs)* [32] and eventually the perpetuation of the process will happen? A vicious cycle feeding an excessive immune activation is likely a common early event in the process of autoimmunity.

2- Why all PBC patients do not have anti-gp210 antibodies?

In the present study, although all PBC patients had AMA, only 29.2% of them had anti-gp210 antibody. Interestingly, the mean frequency of anti-gp210 antibody (30%) found in different studies is similar to that of increased intestinal permeability reported in PBC (25%) [33]. Remarkably, the frequency of anti-gp210 antibodies in the present study (29.2%) is similar to that of anti-*S. cerevisiae* antibodies (ASCA) in PBC (24.2%) in our previous study [34].

Furthermore, our frequency of anti-gp210 antibody was consistent with that of ASCA in PBC patients, as reported by Hu et al. [35] (29.2% and 29.8%, respectively). More interestingly, Hu et al. [35] demonstrated that positive anti-gp210-PBC patients had a significantly higher frequency of ASCA than had negative anti-gp210-PBC patients and suggested that ASCA, such as anti-gp210, might be a prognostic marker. Additionally, they showed that ASCA are present in 46.7% of AMA-negative PBC patients, suggesting that ASCA can be considered as a diagnostic marker for AMA-negative PBC patients [35].

To conclude, it is significant to combine anti-gp210 and anti-Sp100 antibodies in the immunological exploration of PBC. We endeavored to explain why anti-gp210 antibody was produced in PBC. We speculate that anti-gp210 antibodies are both causes and consequences of BEC destruction and that their production is probably initiated by gut microbiota dysbiosis and subsequent leaky gut.

Our study presents some limitations. First, the patients were selected from the database of our laboratory; thus, only the sera of AMA-positive PBC patients are available. It could be interesting to extend the study to AMA-negative patients. Second, another significant value of anti-gp210 antibody is related to prognosis. However, the relationship between anti-gp210 antibody and prognosis was not analyzed in this study. Third, in this study, the production of anti-gp210 is assumed to be due to the dysbiosis of the gut microbiota and intestinal leakage. It would be imperative to consider the results for ASCA in positive and negative anti-gp210 antibody sera.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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