Journal of Autoimmunity 34 (2010) 55–58

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

A comprehensive evaluation of serum autoantibodies in primary biliary cirrhosis

Nancy Agmon-Levin [a,b], Yinon Shapira [a], Carlo Selmi [c], Ori Barzilai [a], Maya Ram [a], Martine Szyper-Kravitz [a,b], Sara Sella [a], Bat-sheva Porat Katz [d], Pierre Youinou [e,c], Yves Renaudineau [e,c], Bruno Larida [f], Pietro Invernizzi [c], M. Eric Gershwin [g], Yehuda Shoenfeld [a,b,h,*]

[a] Center for Autoimmune Diseases Sheba Medical Center, Israel
[b] Department of Medicine 'B', Sheba Medical Center, Israel
[c] Department of Internal Medicine, IRCCS Istituto Clinico Humanitas, University of Milan, Italy
[d] Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Israel
[e] Laboratoire d'immunologie, Brest University Medical School Hospital, Brest, France
[f] Bio-Rad Laboratories, CA, USA
[g] Division of Rheumatology, Allergy and Clinical Immunology, University of California at Davis School of Medicine, Davis, CA, USA
[h] Sackler Faculty of Medicine, Incumbent of the Laura Schwarz-Kip Chair for Research of Autoimmune Diseases, Tel-Aviv University, Israel

ARTICLE INFO

Article history:
Received 19 August 2009
Accepted 24 August 2009

Keywords:
Primary biliary cirrhosis
Anti-prothrombin antibodies
Autoimmune cholangitis
Anti-phospholipid antibodies
Anti-nuclear antibodies

ABSTRACT

In primary biliary cirrhosis (PBC) serum markers other than anti-mitochondrial antibodies (AMA) are promising in terms of disease severity and comorbidities, as well represented by anti-nuclear antibodies (ANA). The aim of the present study was thus to evaluate the prevalence and clinical significance of a large profile of serum autoantibodies in PBC sera. We utilized 69 sera from European patients with PBC (including 20 AMA-negative) and 297 sera from geographically and sex-matched healthy controls. All sera were tested for the presence of ANA and autoantibodies associated with thrombophilia, vasculitis, and gastrointestinal disease. Autoantibodies other than AMA were detected in 53/69 (76%) PBC sera *vs.* 105/297 (35%) among controls. The prevalence of ANA (targeting dsDNA, Sm, chromatin, ribosomal-P, RNP, SmRNP, SSA, SSB, and centromere) and thrombophilia-associated autoantibodies (i.e. anti-β2GPI, phosphatydilserine, prothrombin) was common among patients with PBC. When clinical features were compared, the presence of anti-prothrombin IgM was associated with a worse prognosis as represented by a higher Mayo score. We demonstrate an increased prevalence of ANA and thrombophilia-associated autoantibodies in PBC sera and an association between the latter autoantibodies and PBC stage. The role of thrombophilia-associated antibodies will warrant further studies, based in particular on the incidence of portal hypertension at early stages of PBC.

1. Introduction

Determining the diagnosis and prognosis of primary biliary cirrhosis (PBC) remains challenging. Several authors have attempted to identify non-invasive markers to allow a more accurate diagnosis, particularly when anti-mitochondrial antibodies (AMA) are not detected, or to predict disease severity. Indeed, AMA are the serum hallmark for PBC and this antibody at titres above 1:40 is highly specificity for PBC [1] and can be observed long before the disease is clinically overt [2]. Nevertheless, AMA fail to predict the clinical phenotype or the prognosis of PBC [3] and do not determine clinically different populations [4] nor change during progression [5]. The search for additional serum markers is well represented by anti-nuclear antibodies (ANA) [6] which are associated with disease severity regardless of the AMA status [7]. The identification of new non-invasive markers for disease severity remains a priority in PBC as patients may manifest an indolent or very aggressive disease [8]. Another fascinating issue in PBC is the appearance of portal hypertension at early disease stages, i.e. when no sign of cirrhosis is present [9,10], yet no clear mechanism has been identified for this unique feature among progressing liver diseases.

To address these issues we investigated the autoantibody profile in sera from patients with PBC and performed a cross-sectional analysis of the significance of observed reactivities.

2. Materials and methods

2.1. Subjects

Sera were obtained from 69 European patients with an established diagnosis of PBC [11]; sera were randomly chosen but

* Corresponding author at: Department of Medicine 'B' & Center for Autoimmune Diseases, Chaim Sheba Medical Center, Tel-Hashomer 52621, Israel. Tel.: +972 3 5302652; fax: +972 3 5352855.
E-mail address: shoenfel@post.tau.ac.il (Y. Shoenfeld).

0896-8411/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jaut.2009.08.009

included 20 AMA-negative as determined by indirect immunofluorescence, immunoblotting, and ELISA on recombinant antigens [2]. Patients manifested no significant differences based on their AMA status, as expected [4] (Table 1). Clinical and biochemical features included age, sex, histological evidence of fibrosis or cirrhosis (i.e. stages III–IV [12]), ongoing treatment with ursodeoxycholic acid (UDCA), levels of bilirubin, alkaline phosphatase, prothrombin time INR; these data were ultimately used to calculate the Mayo risk score [13], the only validated prognostic index for PBC. Sera from 297 geographically matched healthy volunteers were used as controls. The study received approval by the local ethical committees and fulfilled the ethical guidelines of the most recent declaration of Helsinki (Edinburgh, 2000).

2.2. Methods

All sera were tested for a panel of autoantibodies (listed in Table 2) using the Bio-Rad BioPlex 2200 system (Bio-Rad Laboratories, Hercules, CA), a fully automated random-access analyzer built on a synthesis of multiplex, magnetic beads and flow cytometry technologies as illustrated elsewhere [14,15]. The profile of autoantibodies included IgG ANA (i.e. dsDNS, Sm, chromatin, ribosomal-P, RNP, SmRNP, Ro/SSA, La/SSB, centromer, Scl-70, and Jo-1); IgG autoantibodies associated with vasculitis (i.e. anti glomerular basement membarane (GBM), proteinase 3 (PR3), and myeloperoxidase (MPO) antibodies); IgG and IgA autoantibodies associated with gastrointestinal autoimmune diseases (i.e. anti-*Saccharomyces cerevisiae*, anti-gliadin, and anti-tissue transglutaminase antibodies); IgG and IgM autoantibodies associated with thrombophilia (i.e. anti-cardiolipin (CL), anti-β2 glycoprotein I (β2GPI), and the complex of both (anti-CL–B2), anti-phosphatydilserine–β2GPI complex (PS–B2), anti-phosphatydilethanolamine (PE), anti-prothrombin (PT), and anti-phosphatydilserine–prothrombin complex (PS–PT)). Serum ANA were also determined using indirect immunoflurescence on Hep-2 cells at different dilutions using FITC-conjugated secondary antibodies (Orthoplan, Wetzlar Germany); titres exceeding 1:40 were considered positive.

2.3. Statistical analysis

Comparison of prevalence rates between groups was preformed by chi square test and Fisher exact test (two tailed), as appropriate. Continuous variables are expressed as mean ± standard deviation throughout the manuscript. Mann–Whitney *U* test was performed for comparison of titer levels between groups. For all tests P values < 0.05 were considered statistically significant and the *StatSoft*-STATISTICA (6.0) program was used for all analyses.

Table 1
Clinical, biochemical, and histological features of AMA-positive and -negative PBC cases.

	AMA-positive ($n = 49$)	AMA-negative ($n = 20$)	*P*
Age (years)	61 ± 11	57 ± 11	*NS*
Sex (female)	96%	100%	*NS*
Total bilirubin (mg/dl)	1.1 ± 0.7	0.7 ± 2	*NS*
Alkaline phosphatase (IU/l)	401 ± 317	326 ± 190	*NS*
Prothrombin time (INR)	0.96 ± 0.3	1 ± 0.06	*NS*
Ongoing UDCA Treatment	67%	70%	*NS*
Mayo score	5.8 ± 0.8	5.5 ± 0.6	*NS*
Stage III–IV	65%	56%	*NS*

Table 2
Autoantibodies prevalence in PBC and control sera.

Autoantibody	PBC patients (no. 69)	Controls (no. 297)	p
Any non-AMA antibody	76%	35%	<0.0001
Anti-nuclear group (IgG):			
Anti-dsDNA	22%	9%	<0.01
Anti-Sm	7%	<1%	<0.01
Anti-Chromatin	25%	3%	<0.001
Anti-Ribosomal P	5%	0%	<0.01
Anti-RNP	8%	1.4%	<0.01
Anti-SmRNP	8%	1.4%	<0.01
Anti-Ro/SSA	10%	2%	<0.01
Anti-La/SSB	7%	1.7%	<0.05
Anti-Centromer	18%	<1%	<0.001
Anti-Scl-70	3%	2.4%	NS
Anti-Jo-1	1.5%	<1%	NS
Vasculitis associated (IgG):			
Anti-GBM	0	0	NS
Anti-PR3	3%	<1%	NS
Anti-MPO	0	<1%	NS
Gastrointestinal associated:			
Anti-tissue transglutaminase (IgG)	4%	1%	NS
Anti-tissue transglutaminase (IgA)	1.5%	1%	NS
Anti-gliadin (IgG)	6%	10%	NS
Anti-gliadin (IgA)	3%	1%	NS
Anti-*Saccharomyces cerevisiae* (IgG)	4%	<1%	0.05
Anti-*Saccharomyces cerevisiae* (IgA)	1.5%	0	NS
Thrombophilia associated:			
Anti-CL (IgG)	0	1%	NS
Anti-β2GPI (IgG)	15%	12%	NS
Anti-CL–B2 (IgG)	1.5%	7%	NS
Anti-PS–B2 (IgG)	0	0	NS
Anti-PE (IgG)	5%	1%	NS
Anti-PT (IgG)	7%	2%	NS
Anti-PS–PT (IgG)	12%	0	<0.001
Anti-CL (IgM)	0	4%	NS
Anti-β2GPI (IgM)	15%	5%	<0.05
Anti-CL–B2 (IgM)	10%	8%	NS
Anti-PS–B2 (IgM)	16%	4%	<0.01
Anti-PE (IgM)	1.5%	4%	NS
Anti-PT (IgM)	27%	13%	<0.05
Anti-PS–PT (IgM)	1.5%	9%	NS

Abbreviations: dsDNA, double strand DNA; Sm, Smith; RNP, ribonucleoprotein; GBM, glomerular basement membrane; PR3, proteinase 3; MPO, myeloperoxidase; CL, Cardiolipin; β2GPI, beta2-glycoprotein I; CL–B2, Cardiolipin-beta2-glycoprotein I complex; PS–B2, phosphatydilserine–beta2-glycoprotein I complex; PE, phosphatydilethanolamine; PT, prothrombin; PS–PT, phosphatydilserine–prothrombin complex; NS, Not significant; $P < 0.05$ was considered significant.

3. Results

3.1. Prevalence of serum autoantibodies

Autoantibodies other than AMA were detected in 53/69 (76%) of PBC sera and in 105/297 (35%) of controls as detailed in Table 2. Autoantibodies directed at nuclear antigens (i.e. anti dsDNS, Sm, chromatin, ribosomal-P, RNP, SmRNP, SSA, SSB, centromer antibodies) and thrombophilia-associated antigents (i.e. β2GPI, phosphatydilserine–β2GPI complex (PS–B2), phosphatydilserine–prothrombin complex (PS–PT) and prothrombin (PT)) were more frequently detected in PBC sera compared to healthy subjects. Other antibodies directed at nuclear and thrombophilic antigens as well as autoantibodies associated with vasculitis (anti-GBM, anti-MPO and anti PR3) and gastrointestinal diseases (anti-gliadin and anti-transglutaminase) were detected in equal percentages among PBC patients and controls. IgG anti-*Saccharomyces cerevisiae* antibodies were detected in 4% of patients with PBC ($P = 0.05$ *vs.* controls).