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Autoantibody profiling of patients with primary biliary cirrhosis using a multiplexed line-blot assay

Danilo Villalta [a,*], Maria Concetta Sorrentino [b], Elia Girolami [c], Marilina Tampoia [d], Maria Grazia Alessio [e], Ignazio Brusca [f], Massimo Daves [g], Brunetta Porcelli [h], Giuseppina Barberio [i], Nicola Bizzaro [j], on behalf of the Study Group on Autoimmune Diseases of the Italian Society of Laboratory Medicine

[a] Allergologia e Immunologia Clinica, A.O. S. Maria degli Angeli, Pordenone, Italy
[b] Laboratorio Analisi Chimico-Cliniche e Microbiologiche, ISMETT, Palermo, Italy
[c] Dipartimento di Oncoematologia Pediatrica e Medicina Trasfusionale, Ospedale Bambino Gesù, Roma, Italy
[d] Patologia Clinica, Policlinico Consorziale di Bari, Italy
[e] Laboratorio Analisi Chimico-Cliniche, Ospedali Riuniti di Bergamo, Italy
[f] Patologia Clinica, Ospedale Buccheri La Ferla, Palermo, Italy
[g] Laboratorio Centrale, Ospedale Civile, Merano, BZ, Italy
[h] Dipartimento di Biotecnologie Mediche, Università di Siena, Italy
[i] Patologia Clinica, Ospedale Cà Foncello, Treviso, Italy
[j] Patologia Clinica, Ospedale San Antonio, Tolmezzo, UD, Italy

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ABSTRACT

Objective: To evaluate the autoantibody profile in patients with primary biliary cirrhosis (PBC) using a new multiplexed line-blot assay specifically designed for the diagnosis of autoimmune liver diseases.
Methods: Sera of 58 consecutive PBC patients and 191 disease controls (144 with autoimmune liver diseases other than PBC, and 67 with non-autoimmune chronic liver diseases) were tested by both the multiplexed line-blot Autoimmune Liver Disease Profile 2 (ALD2) and by IIF on HEp-2 cells and on rat kidney/liver/stomach tissues. ALD2 contains the following PBC-associated antigens: AMA-M2, natively purified from bovine heart; M2-E3, a recombinant fusion protein including the E2 subunits of PDC, BCOADC and OGDC; sp100, PML and gp210 recombinant proteins.
Results: With the ALD2 assay, a positive reaction to AMA-M2, M2-E3, sp100, PML and gp210 in PBC patients was observed in 77.6%, 84.5%, 34.5%, 15.1% and 18.9%, respectively, of the PBC sera. The overall sensitivity and specificity for PBC were 98.3% and 93.7%. Using IIF, positivity rates to AMA, and to antinuclear autoantibodies with membranous/rim-like and multiple nuclear dot patterns were 86.2%, 8.6% and 22.4%, respectively. The overall sensitivity and specificity for PBC of the IIF method were 86.2% and 97.9%, respectively.
Conclusions: The ALD2 line-blot showed a good diagnostic accuracy for PBC and a higher sensitivity than the IIF method to detect sp100 and gp210 autoantibodies.

1. Introduction

Primary biliary cirrhosis (PBC) is an autoimmune liver disease characterized by the progressive destruction of the small intra-hepatic bile ducts, resulting in cholestasis, portal inflammation, and fibrosis that may lead to cirrhosis and over time to liver failure [1]. Anti-mitochondrial autoantibodies (AMA) detected by the indirect immunofluorescence (IIF) method on rodent kidney/liver/stomach tissues are the classical specific serological markers of PBC and are found in 90–95% of PBC patients [2]. Due to their high diagnostic value, the presence of AMA represents one of the three criteria required for a definite diagnosis of PBC [3–5].

Berg et al. [6] showed that most of the PBC-specific AMA bind in vitro to a trypsin-sensitive antigen of the inner mitochondrial membrane, and this antigen was later designated M2. Subsequently, the target antigens of AMA-M2 were identified as components of the 2-oxo-acid dehydrogenase complex catalyzing the oxidative decarboxylation of keto-acids [7]. More specifically, the autoantibodies are mainly directed to the E2 subunits of the 2-oxo-acid dehydrogenase complex (pyruvate dehydrogenase complex [PDC-E2], 2-oxoglutarate dehydrogenase complex [OGDC-E2], branched-chain 2-oxoacid dehydrogenase complex

* Corresponding author at: Allergologia e Immunologia Clinica, A.O. S.Maria degli Angeli, Via Montereale 24, 33170 Pordenone, Italy. Tel.: +39 0434 399647; fax: +39 0434 399344.
E-mail address: danilo.villalta@aopn.sanita.fvg.it (D. Villalta).

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[BCOADC-E2]), and to a lesser extent to the E1 and E3 subunits. Up to 90% of AMA-M2 antibodies are directed against PDC-E2, whereas about 50% react with OGDC-E2 and BCOADC-E2 [8].

Besides AMA, PBC-specific antinuclear autoantibodies (ANA) with membranous/rim-like and multiple nuclear dots (MND) IIF patterns are detected in approximately 25–30% of patients. In the membranous/rim-like pattern the autoantibodies are directed against gp210 and nucleoporin-62 antigens, while in the MND pattern they are directed against sp100, sp140, promyelocytic leukemia nuclear body proteins (PML) and ubiquitin-like modifier (SUMO) proteins. The detection of these PBC-specific ANA can be used to confirm the diagnosis of PBC in AMA-negative cases, thus increasing the sensitivity of serological tests in diagnosing PBC. Moreover, some authors showed that these ANA are associated with a more severe disease course [9–11].

In addition, other non-PBC-specific ANA, especially those displaying speckled or anti-centromere IIF patterns, may be found in a subgroup of patients, suggesting the presence of a concurrent autoimmune disease (i.e. systemic sclerosis in patients with anti-centromere antibodies) [12].

Following the identification of the AMA and of the PBC-specific ANA target antigens, a number of different analytical methods such as ELISA, dot- or line-blot, or fluorescent bead-based assays, have been developed. These methods use either a mixture of recombinantly produced human E2 subunits [13], or a fusion protein combining all the three E2 subunits [14], or a mixture of native and recombinant antigens [15]. The aim of our study was to evaluate the autoantibody profile in a cohort of consecutive PBC patients and in patients with other autoimmune and non-autoimmune liver diseases, using a new multiplexed line-blot assay designed for the diagnosis of autoimmune liver diseases.

2. Patients and methods

Sera obtained at the diagnosis of 58 consecutive PBC patients diagnosed following the internationally accepted criteria [1,3,4] (mean age, 59.8 years; range, 37–81; male/female ratio, 1:4.3) and 191 disease controls (133 autoimmune hepatitis [AIH], 11 primary sclerosing cholangitis [PSC] diagnosed by established contrast cholangiography [16] and 67 non-autoimmune chronic liver diseases [including 39 liver steatosis, 11 chronic alcoholic liver diseases, 9 cryptogenetic cirrhosis, and 8 toxic non-alcoholic liver diseases]) (mean age, 41.1 years; range, 1–81 years; male/female ratio, 1:1.85) were collected in 10 different hospital and university centers in Italy.

All the sera were tested with the multiplexed line-blot Autoimmune Liver Disease Profile 2 (ALD2) (Euroimmun, Lübeck, Germany), containing the following PBC-associated antigens: AMA-M2-natively purified from bovine heart, containing the 74 kDa E2 subunit of the PDC; the M2-3E recombinant fusion protein comprising the immunogenic domains of the E2 subunits of PDC, of the BCOADC and of the OGDC (MIT3) enriched with native PDC antigen; sp100 and PML recombinant proteins (which are autoantigens associated with MND reactivity); gp210 recombinant protein, an integral component of the nuclear pore complex. The ALD2 profile contains also some non-PBC-specific nuclear autoantigens (Ro52, SSA, CENP-A, CENP-B, Scl70) and some autoimmune hepatitis- (AIH-) specific autoantigens (LKM1, LC1 and SLA/LP). Line-blot assay was performed following the manufacturer's instruction. Blot strips were digitalized using a camera and band intensities were determined by a computer program (EUROLineScan, Euroimmun). Signal strengths >10 arbitrary units (AU) were considered positive, as recommended by the manufacturer.

All the sera were also tested by IIF on HEp-2 cells and on rat kidney/liver/stomach tissues (Euroimmun) at a starting serum dilution of 1:40. The slides were read visually by two expert operators (D.V. and M.G.A.) who worked independently.

Diagnostic sensitivity and specificity were calculated for each PBC-associated autoantibody. Cohen's kappa with 95% confidence interval (95% CI) was used to evaluate the analytical agreement among results obtained with the IIF methods and with the line-blot assay and between AMA-M2 and M2-3E. MedCalc software (Mariakerke, Belgium) was used for statistical analysis.

The study protocol followed the ethical guidelines of the Declaration of Helsinki and informed consent was obtained from all patients included in the study.

3. Results

The autoantibody profiles of PBC patients and controls are shown in Table 1. Using the IIF method, AMA were positive in 86.2% of PBC patients with a specificity of 97.9%. With the ALD2 assay, reactivity to M2-3E-recombinant fusion protein was higher (84.5%) than that to AMA-M2 natively purified from bovine heart (77.6%). M2-E3 also showed a higher specificity than AMA-M2 (98.4% vs. 97.9%).

Positivities to sp100 (34.5%) and gp210 (18.9%) were higher than the corresponding MND (22.4%) and membranous/rim-like (8.6%) ANA pattern positivities observed with the IIF method. Autoantibodies against PML were always associated to autoantibodies to sp100. Autoantibodies to sp100, gp210 and PML, alone or associated, were found in 6/8 (75%) of AMA-IIF-negative PBC patients. Combining the positivity to AMA-M2/ME-E3, sp100, gp210 and PML, the overall sensitivity of the ALD2 assay for PBC was 98.3% and the specificity 93.7%. Combining the results of the PBC-associated IIF patterns (AMA-IIF on rodent tissues and ANA on HEp-2 cells), the overall sensitivity and specificity were 96.6% and 96.3%, respectively.

Among controls, PBC-specific autoantibodies were detected in 10 patients with AIH using the line-blot assay (three AMA-M2 + M2-3E, one AMA-M2, three sp100, one sp100 + PML, two gp210), and in seven patients using the IIF method (four AMA, two MND and one membranous/rime-like positive). Only one of these patients (AMA-M2

Table 1
Demographic and laboratory features of PBC patients and control subjects.

	PBC	Controls
Number	58	191
Mean age (range)	59.8 (37–81)	41.1 (2–87)
Female	47	124
Male	11	67
Indirect immunofluorescence		
AMA	50 (86.2%)	4 (2.1%) [AIH: 2, unclassified: 1; alcoholic: 1]
Multiple nuclear dots	13 (22.4%)	2 (1.0%) [AIH: 1, PSC: 1]
Rim-like/membranous	5 (8.6%)	1 (1.0%) [AIH: 1]
Multiple line immunoblot assay (ALD2)		
AMA-M2	45 (77.6%)	4 (2.1%) [AIH: 2, PSC: 1, steatosis: 1]
M2-3E	49 (84.5%)	3 (1.6%) [AIH: 2, crypto: 1]
sp100	20 (34.5%)	4 (2.1%) [AIH: 2, steatosis: 1, crypto: 1]
gp210	11 (18.9%)	2 (1.0%) [AIH: 2]
PML	9 (15.1%)	1 (0.5%) [AIH: 1]
AMA-M2/M2-3E alone	28 (48.2%)	
sp100 alone	2 (3.4%)	
gp210 alone	1 (1.7%)	
PML alone	0 (0%)	
AMA-M2/M2-3E + sp100	6 (10.3%)	
AMA-M2/M2-3E + gp210	7 (12.1%)	
AMA-M2/M2-3E + sp100 + gp210	2 (3.4%)	
AMA-M2/M2-3E + sp100 + PML	7 (12.1%)	
sp100 + gp210	1 (1.7%)	
sp100 + PML	2 (3.4%)	
CENP-A	3 (5.2%)	3 (1.6%)
CENP-B	3 (5.2%)	8 (4.2%)
Scl-70	1 (1.7%)	4 (2.1%)
Ro60	5 (8.6%)	2 (1.0%)
Ro52	12 (20.7%)	10 (5.2%)