Autoantibodies in the Diagnosis of Autoimmune Liver Disease

Danutė Speičienė, Valentina Liakina, Algimantas Irnius, Jolanta Naraškevičienė

Clinic of Gastroenterology and Dietetics of Vilnius University, Santariškių 2, LT-2021 Vilnius, Lithuania

The aim of the study was to examine different serum autoantibodies in patients with autoimmune liver diseases and to evaluate their diagnostic implications. We examined 129 patients (110 female and 19 male) with autoimmune liver disease, of them 83 with primary biliary cirrhosis (PBC), 18 with autoimmune hepatitis (AIH), 19 with primary sclerosing cholangitis (PSC) and 9 with PBC–AIH "overlap" syndrome) were examined. Autoantibodies (ANA, SMA, AMA, anti- LKM-1, PCA, p-ANCA, c-ANCA) were analyzed by indirect immunofluorescence (on Hep-2 and rodent tissue slides, Binding Site Kits, Great Britain).

AMA were found in 76 (91.6%) of PBC patients: in 46 (55.4%) as a single marker and in 30 (36.2%), concurrently, with ANA and/or SMA. Seven (8.4%) patients with PBC were AMA-negative. These patients presented single ANA in 5 (6.2%) and single SMA in 2 (2.4%) cases.

In AIH patients ANA (as a single and concurrent marker) was found in 12 (66.7%), single SMA in 4 (22.2%) and anti LKM-1 in 2 (11.1%) patients. On that ground AIH type 1 was diagnosed in 16 and AIH type 2 in 2 patients. The most frequent ANA pattern in PBC patients was nuclear dots and centromere in 9 (33.3%) and 6 (22.2%) cases, respectively, meanwhile AIH patients presented mostly homogeneous and speckled ANA patterns (6 cases each).

Fifteen (78.9%) patients with PSC presented ANCA. C-ANCA were detected in 4 (21%), P-ANCA typical in 5 (26.6%) and P-ANCA atypical in 6 (31.5%) patients.

In the PBC–AIH "overlap" group AMA and ANA together were presented in 7 (77.8%), AMA, ANA and SMA in one, and ANA alone in one patient.

In conclusion: various autoantibodies are very useful in the diagnosis of autoimmune liver disease, but only AMA can be considered to be almost patognomic for PBC. Seroposivity for any other autoantibodies strenghtens the diagnosis of autoimmune liver disease, but their absence does not exclude this diagnosis.

The presence of a certain type of autoantibodies or their combinations serves as a basis for subdivision of AIH subtypes 1 and 2 (when ANA, SMA or LKM-1 are present) and can be used as an additional diagnostic criteria for AIH–PBC "overlap" syndrome (when AMA and ANA are found) or contribute to the diagnosis of AMA-negative PBC (*i.e.* autoimmune cholangitis) when AMA is found negative.

Key words: autoimmune liver disease,autoantibodies, primary biliary cirrhosis, autoimmune hepatitis, primary sclerosing cholangitis

INTRODUCTION

The presence of autoantibodies is the hallmark of any autoimmune condition, including autoimmune liver disease.

Autoantibodies (autoAb) are most widely used in diagnosing the disorders which are thought to have an autoimmune basis: autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and a group of overlapping conditions representing a small number of patients with biliary lesions suggestive of either PBC or PSC, who also have features of AIH.

It is very important to make the correct diagnosis of those conditions, because the clinical manage-

Correspondence to:
Dr. Valentina Liakina, Clinic of Gastroenterology and Dietetics, Santariškių 2, LT-2021 Vilnius, Lithuania.
Tel.: 370-2 36-52-34. Fax: 370-2 36-52-30.

ment and prognosis differ significantly among them and other liver disorders.

Immunological tests, including circulating autoantibodies, together with liver histology (in the case of PSC – cholangiography) serve as a diagnostic criterion for AIH, PBC and PSC (1).

ANA and SMA are a common finding in AIH patients.

ANA react with a number of different nuclear antigens, and these reactions are reflected in different immunofluorescent staining patterns (2, 3). The presence of high-titer AMA is a single diagnostic marker of PBC, and they are found in over 95% of patients with PBC. AMA recognizes a complex of proteins located in the inner mitochondrial membrane and termed M2 (4).

According to currently available data, the estimation of different autoantibodies is very useful in the diagnosis and differential diagnosis of chronic liver disease. Seropositivity for any autoantibody strengthens the diagnosis for a liver disease of presumed autoimmune etiology, but the absence of autoantibodies does not exclude the diagnosis of an autoimmune liver disease. The clinical significance of AMA, ANA, SMA, ANCA autoantibodies as well as different staining patterns of ANA in chronic autoimmune liver disease in Lithuania have not been studied.

The aim of the study was to examine different serum autoantibodies in patients with autoimmune liver disease and to evaluate their diagnostic implication.

PATIENTS AND METHODS

One hundred twenty nine patients (110 female and 19 male) with autoimmune liver disease (83 with PBC, 18 with AIH, 9 with PBC-AIH overlap and 19 with PSC) were examined in the Clinic of Gastroenterology and Dietetics (Vilnius University Hospital "Santariškių klinikos") during a 7-year period 1993–2000 (Table 1).

All conventional biochemical tests (including serum immunoglobulins, circulating immune complexes and cryoglobulins), ultrasonography, liver biopsy and/or ERCP were caried out.

All patients were also tested for antibodies to hepatitis C virus (anti HCV by Abbott EIA 2nd generation test system) and for markers of hepatitis B virus (HBsAg, HBeAg, anti HBe, anti HBc) also by Abbott EIA.

Autoantibodies (ANA, SMA, AMA, anti-LKM-1, PCA, P-ANCA, C-ANCA) were detected by indirect immunofluorescence (on Hep-2 and rodent tissue slides, the Binding Site kits, Great Britain, "Olympus" BX50/Bx40) (5).

Screening dilution for ANCA was 1:20 and diagnostic titer ≥1:40, for other autoantibodies 1:80 and ≥1:80, respectively. Diferent ANA pattern (homogeneous, speckled, nuclear dots, centromere and nuclear rim, as well as C-ANCA, P-ANCA and typical or atypical P-ANCA were evaluated . According to the International Consensus Statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA) (1999), the nomenclature P-ANCA and C-ANCA (instead of p-ANCA and c-ANCA) is used (6).

To ensure test quality, inner (with control sera) and outward ("Binding Site" Quality Assurance Scheme) controls are constantly performed.

Diagnostic criteria

AIH, PBC and PSC were diagnosed in accordance with the established clinical, biochemical, serological and histological criteria (6, 7–9, 10–14).

The overlap syndrome (AIH-PBC) was diagnosed when simultaneous occurrence of AIH and PBC clinical, biochemical, serological and histological features was present (11, 15).

The distribution of PBC and PBC-AIH overlap patients according to clinical PBC stages is presented in Table 2.

In the AIH group seven patients had liver cirrhosis (2 – Child A, 5 – Child B st.). Patients with PBC-AIH overlap had liver cirrhosis Child A in 5, Child B in 4 cases.

According to Ludwig (1998) PSC classification (12), 11 PSC patients had I–II stage, 5 –stage III, and 6 – stage IV of PSC. In 8 patients coexistance of PSC with IBD was found: in 3 patients with ulcerative colitis (UC) and in 5 – unclassified colitis.

Table 1. **Patients**

	PBC		AIH		PSC		PBC/AIH	
	Age (years)							
	n	Median (range)	n	Median (range)	n	Median (range)	n	Median (range)
Male	3	62.3 (57–70)	5	26.4 (11–50)	11	38.0 (17–70)	0	
Female	80	63.1 (29–88)	13	48.7 (25–73)	8	49.8 (29–77)	9	59.1 (43–73)
Total	83	62.3 (29–88)	18	28.0 (11–73)	19	44.4 (17–77)	9	59.1 (43–73)

Table 2. **Stages of cirrhosis in PBC and PBC–AIH "overlap" patients**

	n	Stages					
		I–II	II	II–III	III	III–IV	IV
PBC	83	1	4	9	36	10	23
PBC–AIH (overlap)	9	0	0	4	2	3	0

RESULTS

Prevalence of autoantibodies

Autoantibody profiles in patients with autoimmune liver disease are shown in Table 3.

AMA as a single marker of PBC was found in 46 (55.4%), AMA and ANA concurrently in 26 (31.3%), AMA and SMA in 3 (3.6%), AMA, ANA and SMA in 1 (1.2%) of the PBC patients. So AMA positivity altogether was found in 76 (91.6%) cases. Seven (8.4%) patients with PBC were AMA-negative. In 5 (6.2%) of them single ANA and in 2 (2.4%) SMA positivity (in a titer 1:80–1:160) were assessed.

In AIH patients ANA as a single parameter was found in 4 (22.2%), ANA and SMA in 5 (27.8%), ANA and AMA (the latter in low titer) in 2 (11.1%), ANA and antiperoxisome antibodies in 1, single SMA in 4 (22.2%). Altogether (as a single and concurrent marker) ANA seropositivity was found in 12 (66.7%). Anti-LKM-1 were positive in 2 (11.1%) AIH patients. So, all patients with AIH had a certain type (ANA, SMA, LKM-1 or others) of autoantibodies or their combination.

In the PSC group, C-ANCA were detected in 4 (21.0%), typical P-ANCA in 5 (26.3%) and atypical P-ANCA in 6 (31.5%) patients. So, 15 (78.9%) patients with PSC had different types of ANCA. In 5 (27.7%) patients single ANA and in one patient ANA and SMA were detected, too.

In patients with PBC-AIH "overlap" syndrome AMA and ANA had 7 (77.8%) patients, AMA, ANA, SMA had one and ANA also one patient.

Autoantibody titer (Table 4). Most of PBC and PBC-AIH "overlap" patients (51/61.4% and 5/55.6%) had a low titer 1:80 – 1:160 of AMA. Moderate (1:160 – 1:640) and high titers (≥640) were found

Table 3. **Autoantibody profiles in patients with autoimmune liver disease**

Autoantibodies	PBC (n = 83)	AIH (n = 18)	PSC (n = 19)	PBC/AIH (n = 9)
	n/%	n/%	n/%	n/%
AMA	46/55.4	0	0	0
AMA and ANA	26/31.3	2/11.1	0	7/77.8
AMA, ANA, SMA	1/1.2	0	0	1/11.1
SMA	2/2.4	4/22.2	0	0
SMA and ANA	0	5/27.8	1/5.6	0
SMA and AMA	3/3.6	0	0	0
ANA	5/6.06	4/22.2	5/27.7	1/11.1
LKM	0	1/5.6	0	0
LKM and SMA	0	1/5.6	0	0
Antiperoxisome Ab and ANA	0	1/5.6	0	0
C-ANCA	0	0	4/18.2	0
P-ANCA typical	0	0	5/22.7	0
P- ANCA atypical	0	0	6/27.3	0
AMA neg	7/8.4			

Table 4**. Titers of autoantibodies in patients with autoimmune liver disease**

	Titer	PBC n/%	AIH n/%	PSC n/%	PBC+AIH n/%
AMA	Total (n)	76/91.6	2/11.1	0	8/88.9
	1:80–1:160	51/61.4	2/11.1		5/55.6
	1:160–1:640	19/22.9	0		3/33.3
	≥ 1:640	6/7.2	0		
ANA	Total (n)	27/32.5	12/66.7	0	9/100.0
	1:80–1:160	20/24.1	9/50.0		5/55.6
	1:160–1:640	7/8.4	3/16.7		4/44.4
	≥ 1:640	0	0		
SMA	Total (n)	5/6.0	10/55.6	0	0
	1:80–1:160	5/6.0	8/44.4		
	1:160–1:640	0	2/11.1		
	≥ 1:640	0			
LKM-1	Total (n)	0	2/11.1	0	0
	1:80–1:160	0	2/11.1	0	

in 19 (22.9%) and 6 (7.2%) PBC patients, respectively.

Two patients with AIH presented AMA in a low titer together with ANA. Neither biochemical nor histological parametres of bile ductule damage were found in those two patients.

Most patients with AIH – 9 (50%) and 8 (44.4%) – had a low titer of ANA and SMA, meanwhile 44.4% of pts with PBC-AIH "overlap" presented ANA in a moderate titer (1:160 – 1:640). Both patients with AIH type 2 had LKM-1 in a low titer (1:80 – 1:160). ANCA in PSC patients were presented in a titer 1:40 – 1:160.

ANA patterns (Table 5)

The most frequent ANA pattern presented by PBC patients was found to be nuclear dots (9, or 33.3%) and centromere (6, or 22.2%), meanwhile AIH patients mostly had homogeneous (6 cases) and speckled (6 cases) patterns.

In PBC-AIH "overlap" patients we didn't find any significant prevalence of a definite ANA patern.

Table 5. **Prevalence of different ANA patterns in patients with autoimmune liver disease**

	homo-geneous	speckled		nuclear dots		centrosome		centromere		nuclear rim	
		alone	with homog	alone	with homog	alone	with homog	alone	with homog	alone	with homog
PBC n = 27	2	3	2	4	5	2	1	6	0	1	1
AIH n = 12	6	5	1	0	0	0	0	0	0	0	0
PBC/AIH n = 9	0	2	0	1	1	1	2	0	1	0	1

DISCUSSION

All autoimmune liver diseases – PBC, AIH, PSC and their "overlaps" (PBC and AIH or PSC and AIH) need precise discrimination by serological tests because of the necessity to provide a rational basis for the application of different therapeutic strategies.

In our study, AMA were found in 76 (91.6%) of PBC patients: in 46 (55.4%) as a single marker and in the other 30 (36.1%) patients concurrently with ANA and/or SMA. The low titer of AMA (1:80 – 1:160) was detected in 51 (61.4%), moderate in 19 (22.9%) and high (≥ 1:640) in 6 (7.2%) PBC patients. Seven (8.4%) patients with PBC were AMA-negative.

Primary biliary cirrhosis (PBC) is a chronic autoimmune liver disease characterized by inflammation and destruction of the small intrahepatic bile ducts. The presence of high-titer AMA in patient's sera constitutes the single diagnostic marker of PBC. AMA recognizes a complex of proteins located in the inner mitochondrial membrane and termed M2 (4). In 1985 subclassification of M2 into three major antigens was carried out: pyruvate dehydrogenase (PDH-E2, 74 kDa), branched chain ketoacid dehydrogenase (BCKD-E2, 52 kDa) and ketoglutarate dehydrogenase (KGDH-E2, 48 kDa) (3, 16–21). These antibodies mainly belong to the IgM but also to the IgG1 and IgG3 subgroups (2). It has been suggested in the past that AMA simply arise in PBC patients as a response to hepatocytes and bile ductuli injury. But recent data support the concept that PBC is indeed an organ-specific autoimmune disease and that those major mitochondrial autoantigens are recognized by AMA. So, they have a pathogenetical significance (2).

Diagnosis of PBC usually is based on cholestatic liver tests, AMA positivity and diagnostics or compatible liver histology. But the most important criterion, hallmark of PBC diagnosis is AMA positivity. In about 90–95% of patients with PBC autoantibodies against mitochondrial antigens are presented and considered the signature of PBC (2, 3).

Estimation of AMA is particularly valuable in a very early "preclinical" PBC phase, when still there are no clinical and biochemical abnormalities and in asymptomic phase (10–15% of PBC patients), when laboratory tests already reveal cholestasis (11, 13, 22, 23).

The early PBC diagnosis is very important for the management of this disease. It was proved that UDCA improved survival free of transplantation, reduced serum lipid level, particularly cholesterol, reduced the risk of developing varices, retarded the histologic progression to cirrhosis (24, 25). So, early administration of UDCA may improve the prognosis of PBC patients and their quality of life.

It has been long recognized that aproximately 5–10% of patients with clinical, histological and biochemical features typical of PBC are AMA-negative.

Brunner and Klinge in 1987 (26) suggested the term of autoimmune cholangitis (AIC) and defined it as an inflammatory bile duct disease with the presence of ANA. This definition is still discussed and sometimes modified: or discribes AIC as a part of AIH type 1 (27), or as an AMA- negative form of PBC (28). Strassburg and Manns (2) reported a case which clearly demonstrate that AIC may be a disease of coexistence of two entities (AIH and PBC) or the progression of one disease (PBC) to the next (AIH/AIC). Such terminology led to some confusion with PSC, in which similar autoantibody abnormalities can be found but which has distinct histological and cholangiographic manifestations. So, Heathcote (1997) suggested the term AMA-negative PBC, rather than those including cholangiopathy or cholangitis. Several studies found no significant differences between AMA-positive and AMA-negative patients in respect to sex, age, clinical symptoms or histology (2, 3, 14, 29). At the same time in AMA-negative patients the serum IgM concentration was significantly lower than in AMA-positive ones (14). In each of those patients had the serum was also positive for ANA, usually in high titer (>1:160), whereas only 17.6% of AMA-positive patients were ANA-positive. Patients of AMA-negative group were more positive for SMA than were AMA-positive patients (41.2% *vs* 5.9%) (2, 28).

Strassburg and Manns (2) reported that ANA and SMA are serological markers that occur in 91% and 45% of patients, respectively, and contribute to the diagnosis of autoimmune cholangitis.

Our data concerning ANA and SMA positivity in AMA-negative PBC patients or patients with autoimmune cholangiopathy are very similar to those reported above: ANA positivity (in a titer 1:80 – 1:320) was found in 5 and SMA in 2 of 7 AMA-negative PBC patients.

ANA in PBC patients mostly presented in two different "multiple nuclear dot" (*i.e.* SP 100) and nuclear rim-line (gp 210) patterns (10). The difference between these two patterns was discovered to be a recognition of intranuclear proteins *versus* that of the nuclear envelope.

The punctate staining of the nuclear envelope corresponds to antibodies that recognize nuclear pore complexes (30) and are found in about 25% of AMA-positive patients with PBC, but is close to 100% in the AMA-negative patients. According to Strassburg and Manns (2), ANA as a serological parameter were found in up to 52% of patients with PBC. Our data correspond to these findings.

Circulating autoAb as well as clinical, biochemical and histological features were reassessed as the diagnostic criteria of AIH by a panel of experts (The International Autoimmune Hepatitis Group). Subdivision of AIH to 3 types 1, 2 and based on marker autoAb is reasonable and justified by differences in some clinical features and prognosis of patient populations (7, 8, 31).

AIH type 1 characterized by ANA and SMA is the common type of AIH accounting for about 60–70% of patients (32). In about 20% of patients of this group low titers of AMA are found too. AIH type 2 is characterized by autoAb against microsomal antigens of liver and kidney (LKM-1) in the absence of ANA and SMA. AIH type 2 is a rare disease accounting for only 5% of patients with AIH. AIH type 3 represents a poorly defined group of patients with presence of autoAb against SLA, LP, ASGPR, etc (7, 8, 31, 32).

We found ANA as a sole marker of autoimmune hepatitis in 2 (11.8%) patients and SMA in 4 (23.5%). In 4 patients (23.5%) they were detected concurrently. In two patients (11.8%) an ANA and AMA (in low titers) combination was found. So, in 12 patients (66.6%) with AIH ANA and/or SMA or AMA were found and AIH type 1 was diagnosed. Anti LKM1 were disclosed in 2 (11.8%) patients of this group and diagnosis of AIH type 2 was made.

AIH type 1 as classical AIH is marked by circulating antibodies to nuclei and smooth muscle. The latter is thought to be reflective of the more specific antiactin antibodies. Occasionally, antimitochondrial antibodies occur concomitantly with these autoantibodies, but the isolated presence of AMA signifies PBC, except in the some instances in which an "overlap" syndrome occurs.

Low titers of AMA are found in about 20% of this group withought significant biliary changes when they are investigated by histopathologically or by cholangiography. We found AMA in 2 patients with AIH type 1. They didn't have any clinical or histological lesions of biliary ductules.

It is remarkable that ANA in AIH had mostly another type of pattern when compared with ANA in PBC.

The homogeneous (6 cases) and speckled (6 cases) patterns were most frequent in our patients.

The presence of ANA and AMA represents the situation in which an overlap syndrome – PBC and AIH is likely.

The "overlap" syndrome in autoimmune liver disease designates the simultaneous occurrence of AIH and PBC with clinical and biochemical evidence for autoimmune hepatitis with autoantibodies. Additionally, signs of cholestasis and AMA type 2 have to be present. Histopathology must show criteria of substantial necroinflammatory lesions of liver parenchyma, piecemeal necrosis for the AIH component and portal inflammation, bile duct damage with loss of epithelia and discruption of basement membranes for PBC (15, 22).

Of adult patients with AIH, 8% have a true "overlap" syndrome with PBC. On the other hand, 19% of patients with PBC have signs of AIH (33). The overlap of PBC and AIH is characterized by the presence of ANA in 67% and SMA in 67% (2).

The PBC-AIH "overlap" was diagnosed in 9 (10.8%) of PBC patients. The clinical, biochemical and histological examination revealed symptoms and signs typical of both diseases.

The autoantibody profile was also characteristic of both PBC and AIH: AMA and ANA (both) were found in 7 patients (77.8%), AMA, ANA and SMA in 1, ANA in 1 patient.

The antineutrophil cytoplasmic antibodies (ANCA) have been indentified as markers of systemic vasculitis and were considered a sensitive and specific marker of Wegener's granulomatosis or polyarteritis nodosa, in which main target antigens are myeloperoxidase and proteinase (1, 34)

Two main patterns of staining are recognized: a cytoplasmic pattern (C-ANCA) and a perinuclear pattern (P-ANCA). The C-ANCA reacts with proteinase 3, while P-ANCA recognizes myeloperoxidase and several other antigens (1, 35).

In our study ANCA were found in 15 (78.9%) patients with PSC: 4 (21.0%) had C-ANCA and 11 (57.8%) P-ANCA (5/26.3% typical and 6/31.6% atypical).

According to literature data, P-ANCA are detected in the serum of 26–90% of PSC patients (2, 9, 36). These conflicting data may be attributed to different techniques used for detection of ANCA (37).

Although highly associated with PSC, P-ANCA are not disease-specific. Sera of patients with AIH type1 show positive reactions in about 15% and patients with PBC are positive in 13–18% (36).

The ANCA found in PSC and in inflammatory bowel disease are directed against other targets compared with systemic vasculitis: they show perinuclear immunofluorescence (candidate targets are lactoferin, elastase and cathepsin). The ANCA antigens in PSC and inflammatory bowel disease (IBD) are localized to the granulocyte inner nuclear membrane, and this result indicates that P-ANCA may in fact be an antinuclear antibody directed against granulocyte-specific nuclear lamine proteins. PSC is associated with a variety of autoantibodies: in addition to P-ANCA (70–84%), ANA and SMA (in low and moderate titers) in 35% and 55% of adults were found, respectively. The presence of P-ANCA points to the coexistence of PSC and IBD. This may in future be a useful marker to contribute the identification of a risk of colon carcinoma in this group of patients (5). P-ANCA, however, are not disease-specific, since they occur in a high titer in the majority of patients with AIH type 1 and in the minority of PBC patients (8, 38). So, the impact of P-ANCA as a reliable diagnostic marker of PSC still relies on typical cholangiographic and histological findings, cholestatic profile of liver tests and coexistence with inflammatory bowel disease.

To summarize, estimation of different autoantibodies is very useful in the diagnosis of autoimmune liver disease. AMA can be considered to be most specific and almost patognomic for PBC. ANA and SMA as well as LKM-1 are the diagnosic criteria for AIH and can be used to define patients as having "type1" or "type 2" of AIH. The absence of AMA and presence ANA or SMA in PBC patients is a diagnostic criterion for subdivision of AMA-negative PBC (or autoimmune cholangitis). The presence of AMA and ANA or SMA can be used as an additional (together with biochemical, histological and immunological data) criterion for diagnosis of AIH–PBC "overlap" syndrome. A distinct diagnosis of autoimmune liver disease and "overlaps" is very important, because their clinical management and prognosis differ significantly.

Received 25 March 2000
Accepted 12 November 2000

References

1. MC Farlane I. Autoantibodies in the diagnosis of liver disease: a practical approach. Hepatol Pol 1997; 4 (2) : 95–8.
2. Strassburg CP and Manns MP. Serological tests in cholestatic liver disorders: PSC, PBC and autoimmune cholangiopathy. In: Diseases of the Liver and the Bile Ducts. New aspects and Clinical Implications.Proceedings of the Falk Symposium 107 held in Prague, Czech Respublic, September 12–13, 1998. Dordrecht/ Boston/London : Kluwer Academic Publishers 1999: 183–92.
3. Migliaccio CT, Van de Vater J, Ansari AA et al. Antimitochondrial antibodies are the signature of primary biliary cirrhosis: deciphering the handwriting. In: Cholestatic Liver Diseases. Proceedings of the Falk Symposium 102 (Part I of the Liver Week Freiburg 1997) held in Freiburg, Germany, October 1–2, 1997. Dordrecht– Boston–London: Kluwer Academic Publishers 1998: 239–47.
4. Berg P, Klein R. Mitochondrial antigen/antibody systems in primary biliary cirrhosis: revisited. Liver 1995; 15: 281–92.
5. Broome U, Glaumann H, Hellers G, Nilsson B, Sörstad J, Hultcrantz R. Liver disease and ulcerative colitis. An epidemiological and follow-up study in the county of Stockholm. Gut 1994; 35: 84–9.
6. Savige J, Gilis D,Benson E, Davies D, Esnault V, Falk R et al. International Consensus Statement on Testing and Reporting of Antineutrophil CytoplasmicAntibodies (ANCA). Am J Clin Pathol 1999; 111: 507–13.

7. International Autoimmune Hepatitis Group Report: Review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999; 31: 929–38.
8. Czaja A. The variant forms of autoimmune hepatitis. Ann Intern Med 1996; 125: 588–98.
9. Boberg KM, Schrumpf E. Primary sclerosing cholangitis: diagnosis and differential diagnosis. In: Primary sclerosing cholangitis. Proceedings of the International Falk Workshop (Part II of the Liver Week in Freiburg 1997) held in Freiburg, Germany, October 3, 1997. Dordrecht–Boston–London: Kluwer Academic Publishers 1998: 3–13.
10. International Hepatology Informatics Group. In: "Diseases of the liver and biliary tract". New York: Raven Press 1994: 65–6.
11. James O. Definition and epidemiology of primary biliary cirrhosis In: Primary biliary cirrhosis. Eastburne: West End Studios Ltd. 1999: 53–9.
12. Ludwig J. Histopathology of primary sclerosing cholangitis. In: Primary Sclerosing Cholangitis. Proceedings of the International Falk Workshop (Part II of the Liver Week in Freiburg 1997) held in Freiburg, Germany, October 3, 1997. Dordrecht–Boston–London: Kluwer Academic Publishers 1998: 14–21.
13. Mc Gill JM, Kwiatkowski AP. Cholestatic liver diseases in adults. Am J Gastroen 1998; 93: 684–91.
14. Riordan SM, Williams R. Clinical patterns and natural history of primary biliary cirrhosis. In: Primary biliary cirrhosis. Eastburne: West End Studios Ltd. 1999: 65–74.
15. Dienes HP, Gerken G, Lohse A, Schirmacher P, Meyer K zum Büschenfelde. Morphological aspects of overlap syndromes. In: Autoimmune Liver Disease. Proceedings of The International Falk Workshop held in Konstanz, Germany, January 23–24, 1997. Dodrecht–Boston–London: Kluwer Academic Publishers 1997: 80–5.
16. Frazer I, Markey I, Jordan T. Reactivity of antimitochondrial autoantibodies in primary biliary cirrhosis: definition of two novel mitochondrial polypeptide autoantigens. J Immunol 1985; 135: 1739–45.
17. Surh C, Coppel R, Gershwin M. Comparative epitope mapping of murine monoclonal and human autoantibodies to human PDH-E2, the major mitochondrial autoantigen of primary biliary cirrhosis. J Immunol 1990; 144: 2647–52.
18. Gershwin ME, Mackay IR. Primary biliary cirrhosis: paradigm or paradox for autoimmunity. Gastroenterology 1991; 100: 822–3.
19. Coppell R, McNeilage L, Surh C, Van der Water J, Spithill T, Whittingham S, Gershwin M. Primary structure of the human M2 mitochondrial autoantigen of primary biliary cirrhosis:dihydrolipoamide acetyltransferase. Proc Natl Acad Sci USA 1988; 85: 7317–20.
20. Tanaka A, Munoz S, Van de Water J, Coppel R, Ansari A, Gershwin M. Etiological considerations of primary biliary cirrhosis. In: Primary biliary Cirrhosis. Eastburne: West End Studios Ltd. 1999: 27–38.
21. Howard M, Fuller CK, Broadhurst RW, Perham RN, Jian-Guo Tang, Quinn J et al. Three-dimensional structure of the major autoantigen in primary biliary cirrhosis. Gastroenterology 1998; 115: 139–46.
22. Chapman R. Aetiopathogenesis of primary biliary cirrhosis. In: Primary Biliary Cirrhosis. Eastburne: West End Studios Ltd. 1999: 61–4.
23. Mayer KP. PBC/PSC: Natural course and long-term autcome. In: Autoimmune Liver Disease. Proceedings of the International Falk Workshop held in Konstanz, Germany, January 23–24, 1997. Dordrecht–Boston–London: Kluwer Academic Publishers 1997: 73–9.
24. Keith Lindor D. Ursodeoxycholic acid treatment of PBC: alone and in combination. In: Primary Biliary Cirrhosis. Eastburne: West End Studios Ltd. 1999: 123–7.
25. Leuschner M, Dietrich C, You T, Seidl C, Raedle J, Herrmann G et al. Characterisation of patients with primary biliary cirrhosis responding to long-term ursodeoxycolic acid treatment. Gut 2000; 46: 121–6.
26. Bruhner G, Klinge O. Ein der chronic-destruierenden nichtteitrigen Cholangitis Ähnliches. Krankheitsbild mit Antikörpern (Immuncholangitis) Dtch Med Wochenschr 1987; 112: 1454–8.
27. Ben Ari Z, Ohillon A, Sherlock S. Autoimmune cholangiopathy: part of the spectrum of autoimmune chronic active hepatitis. Hepatology 1993; 18: 10–5.
28. Michieletti P, Wanles I, Katz A, Scheuer P, Ylaman S, Barsendine M et al. Antimitochondrial antibody negative primary biliary cirrhosis : a distinct syndrome of autoimmune cholangitis. Gut 1994; 35: 260–5.
29. Czaja A. The variant forms of autoimmune hepatitis. Ann Intern Med 1996; 125: 588–98.
30. Courvalin JC, Wormen UJ. Nuclear envelope protein autoantibodies in primary biliary cirrhosis. Sem Liv Dis 1997; 17: 71–8.
31. Johnson P, Mc Farlane I. Meeting Report: International Autoimmune Hepatitis Group. Hepatology 1993; 18: 998–1005.
32. Meyer zum Büschenfelde KH. Immunological and clinical aspects of autoimmune hepatitis. In: Autoimmune Liver Disease. Proceedings of the International Falk Workshop held in Konstanz, Germany, January 23–24, 1997. Dordrecht–Boston–London: Kluwer Academic Publishers 1997: 62–72.
33. Czaja AJ. Frequency and nature of the variant syndroms of autoimmune liver disease. Hepatology 1998; 28: 360–5.
34. Beuers U, Paumgartner G. Pathogenesis and clinical spectrum of primary sclerosing cholangitis. In: Diseases of Liver and the Bile ducts. New Aspects and Clinical Implications.Proceedings of the Falk Symposium 107 held in Prague, Czech Respublic, September 12–13, 1998. Dordrecht–Boston–London: Kluwer Academic Publishers 1999: 106–12.
35. Fulcher DA. Anti-neutrophil cytoplasmic antibodies in hepatobiliary disease. J of Gastrenterol and Hepatol 2000; 15: 344–5.
36. Hopf U, Berg T, Körber J, Klein R, Berg P. Autoimmune markers in primary sclerosing cholangitis. In: Primary Sclerosing Cholangitis. Proceedings of the International Falk Workshop (Part II of the Liver Week in Freiburg 1997) held in Freiburg, Germany, October 3, 1997. Dordrecht–Boston–London: Kluwer Academic Publishers 1998: 46–54.

37. Wang G, Csernok E, de Groot K, Gross W. Comparison of eight commercial kits for quantification of antineutrophil cytoplasmic antibodies (ANCA). J Immunol Meth 1997; 208: 203–11.
38. Czaja AJ. Behavior and significance of autoantibodies in type 1 autoimmune hepatitis. J Hepatol 1999; 30: 394–401.

D. Speičienė, V. Liakina, A. Irnius, J. Naraškevičienė

AUTOANTIKŪNAI AUTOIMUNINIŲ LIGŲ DIAGNOSTIKOJE

S a n t r a u k a

Straipsnyje pateikiami autoimuninėmis kepenų ligomis sergančiųjų serumo autoantikūnų (prieš mitochondrijas – AMA, prieš branduolius –ANA, prieš lygiuosius raumenis –SMA, prieš neutrofilų citoplazmos baltymus –ANCA (P-ANCA- perinukleariniai antineutrofiliniai antikūnai ir C-ANCA-citoplazminiai autineutrofiliniai antikūnai) prieš peroksisomas, prieš parietalines skrandžio ląsteles – PCA) duomenys, įvertinama jų diagnostika.

Tyrimai atlikti netiesioginės imunofluorescencijos metodu (naudojant Hep-2 ląsteles ir graužikų audinių pjūvius; „The Binding Site“ Kit's, Didžioji Britanija).

Ištirti 129 ligoniai (110 moterų ir 19 vyrų), sergančių įvairiomis autoimuninėmis kepenų ligomis: 83 sirgo pirmine bilijine kepenų ciroze (PBC), 18 – autoimuniniu hepatitu (AIH), 19 – pirminiu sklerozuojančiu cholangitu (PSC), o devyniems buvo diagnozuota PBC ir AIH derinys. Septyniasdešimt šešiems (91,6%) sergantiesiems PBC buvo rasti AMA titrai (1:80 – ≥640) : 46 (55,4%), kai buvo vienintelis serologinis žymuo; 30 (36,1%) kartu buvo rasti ANA ir/ ar SMA. Dažniausiai pasitaikė nedideli AMA titrai (1:80–1:160) – 51 (61,4%), rečiau – vidutiniai (1:160–1:640) 19 (22,2%), aukšti – (≥1:640) – 6 (7,2%). Septyni šia liga sergantys ligoniai buvo AMA negatyvūs. Penkiems (6,2%) iš jų rasti ANA, dviem (2,4%) – SMA (titras 1:80–1:160). Dažniausiai pasitaikantys ANA švytėjimo tipas – branduolio punktatų centromerinis švytėjimas.

Tarp ligonių AIH ANA kaip vienintelis žymuo buvo nustatytas 4 (22,2%), ANA ir SMA – 5 (27,8%), SMA – 4 (22,2%), ANA ir AMA – 2 (11,1%), ANA ir antiperoksisominiai antikūnai 1 ligoniui. Taigi ANA, kaip vienintelis žymuo ir derinys su SMA, buvo nustatytas 12 (66,7%) AIH grupės ligonių. Remiantis šiais serologiniais žymenimis (kartu su klinikiniais duomenimis), jiems diagnozuotas 1 tipo AIH. Dviem (11,1%) ligoniams rasti LKM-1 antikūnai ir diagnozuotas antro tipo AIH. Dažniausiai pasitaikė homogeninis ir „margas“ (speckled) ANA švytėjimo tipai. Penkiolikai (78,9%) sergančiųjų PSC buvo rasti ANCA: 4 (21%)-C-ANCA, penkiems – (26,3%) – tipiška P-ANCA ir šešiems (31,5%) – atipiška P-ANCA. Šešiems (33,3%) šios grupės pacientams buvo rasti ir ANA, ir SMA.

Tarp PBC-AIH deriniu sirgusių ligonių septyniems (77,8%) buvo rasta tiek AMA, tiek ANA, vienam – AMA, ANA, SMA ir vienam – ANA.

Gauti duomenys patvirtina autoantikūnų svarbą diagnozuojant atskiras imunines kepenų ligas, išaiškinant jų derinius bei diferencijuojant jas. Tai ypač reikšminga parenkant tikslingą gydymo taktiką ir nustatant ilgalaikę ligonių prognozę.