Title: Clinical significance of autoantibodies in PBC

Author: Minoru Nakamura, MD, PhD

Affiliation: Department of Hepatology, Nagasaki University Graduate School of Biomedical Sciences, Clinical Research Center in National Hospital Organization (NHO) Nagasaki Medical Center , Omura, Nagasaki, Japan.

Address: 2-1001-1, Kubara, Omura, Nagasaki 856-8562, Japan.

Tel: +81-957-52-3121, FAX: +81-957-53-6675,

E-mail: nakamuram@nmc.hosp.go.jp

Summary (140 words)

Anti-mitochondrial, anti-gp210, anti-sp100, and anti-centromere antibodies are specifically detected in PBC. In clinical practice, they are useful for the diagnosis of PBC or for evaluating disease severity, clinical phenotype, and long-term outcome. In the typical or classical form of PBC which shows slow progressive loss of small bile ducts with a parallel increase in liver fibrosis, anti-gp210 antibodies are a strong risk factor for progression to jaundice and hepatic failure, while the presence of anti-centromere antibodies is a risk factor for progression to cirrhosis and portal

hypertension. Of note, the autoimmune repertoire, which is established during the early stage of the disease process, can influence the clinical phenotype and the long-term prognosis of PBC. Since the natural course of PBC is being altered by treatment with ursodeoxycholic acid, the clinical significance of these PBC-specific autoantibodies awaits re-evaluation in various ethnicities.

Key words

Anti-mitochondrial antibodies (AMAs)

Anti-nuclear antibodies (ANAs)

Anti-gp210 antibodies

Anti-centromere antibodies (ACAs)

Primary biliary cirrhosis (PBC)

I. Introduction

Primary biliary cirrhosis (PBC) is a chronic, progressive cholestatic autoimmune liver disease characterized by destruction of intrahepatic bile ducts, portal hypertension, and development of cirrhosis and hepatic failure **[1]**. Although the prognosis of PBC has improved markedly since the introduction of ursodeoxycholic acid (UDCA), disease

progression remains highly variable. Approximately 10 to 20% of patients do not respond to treatment and eventually progress to end-stage hepatic failure **[2,3,4]**.

More than 60 distinct autoantibodies have been identified in PBC **[5-9]**. Some of them are specific to PBC and are useful not only for the diagnosis of PBC, but also for the assessment of disease severity, clinical phenotype, and long-term prognosis **(Table 1) [5-11]**. Some autoantibodies are specific for concomitant autoimmune diseases such as Sjogren's syndrome, chronic thyroiditis, systemic sclerosis (SSc), and autoimmune hepatitis (AIH) **[5-7]**. Other autoantibodies seem to be less important in clinical practice because of their low specificity or low sensitivity **[6,7,8]**.

In the present review article, we focus on several PBC-specific autoantibodies, including anti-mitochondrial antibodies (AMAs) and anti-nuclear antibodies (ANAs) in a nuclear envelope (NE) staining pattern, multiple nuclear dot (MND) pattern, and centromere pattern (CENP) **(Table 1 and Figure 1).** We discuss their clinical significance in association with disease phenotype and long-term outcome **[12]**.

II. Anti-mitochondrial antibodies (AMAs)

AMAs are highly PBC-specific autoantibodies that have been detected in

more than 90% of PBC patients but less than 1% of normal controls **(Table 1 and Figure 1A, 1B) [5,6,7]**. AMA-positivity constitutes one of the three major diagnostic criteria for PBC **[2,3,4]**.

The autoantigens targeted by AMAs consist of E2 components of the 2-oxoacid dehydrogenase family of enzyme complexes (2-OACD), including pyruvate dehydrogenase complex (PDC-E2), branched chain 2-oxoacid dehydrogenase complex (BCOADC-E2), 2-oxo-glutarate dehydrogenase complex (OGDC-E2), the E1α subunit of PDC, and E3 binding protein (protein X) **[7,13-16]**. These enzymes are located on the inner mitochondrial membrane and catalyze the oxidative decarboxylation of keto-acid substrates **[7]**. In PBC, PDC-E2 is the primary autoantigen to which more than 90% of the antibodies in the patient's serum react **[7]**. In addition, approximately 50–80% of PBC patients have serum that reacts to BCOADC-E2, 20–60% react to OGDC-E2, 5–25% react to E1α, and 10% react to E3 binding protein **(Table 1) [13-16]**.

Various methods have been used for detecting AMAs, including IIF using sections of rat kidney, stomach, or liver or HEp-2 cells, as well as immunoblotting using purified or recombinant mitochondrial antigens **[17-20]**. To increase the sensitivity of AMA detection, sensitive ELISA systems have been developed using M2 antigens (recombinant PDC-E2), M3 antigens (recombinant proteins co-expressing the

immunodominant epitopes of PDC-E2, BCOADC-E2, and OACD-E2), and a sensitive bead assay **[17,18,19]**. With advances in the methods for detecting AMAs, the proportion of AMA-negative PBC patients has dropped below 5%. Furthermore, approximately half of AMA-negative PBC patients are positive for at least one of the three PBC-specific ANAs, either anti-gp210, anti-SP100, or anti-promyelocytic leukemia (PML) antibodies **[20,21,22]**.

The prevalence of AMAs in the first-degree relatives of PBC probands is as high as 13.1%, whereas the prevalence is only 1% in controls matched by gender, age, race, and residence, suggesting that environmental and genetic risk factors are involved in the pathogenesis of AMA production **[23]**. In addition, AMAs are detected in the serum years before clinical manifestations or biochemical abnormalities, indicating that the immune response to mitochondrial antigens has been initiated before clinical manifestations of PBC develop **[24,25]**. Although the characteristics of AMAs found in healthy subjects may differ from those found in PBC patients **[26]**, AMA positivity in healthy subjects is potentially a risk factor for future development of PBC **[24,25]**.

AMA titers do not change over time and are not associated with disease severity or progression **[27,28]**. The clinical course of AMA-positive and AMA-negative PBC patients are similar **[29,30,31]**. These findings suggest that AMA is

not useful for monitoring the severity or progression of PBC. On the other hand, there have been some reports indicating some differences between AMA-positive and AMA-negative PBC patients **[32-34]**. A large Japanese retrospective database study suggested that pruritus is less frequently observed in AMA-negative patients as compared to AMA-positive patients **[33]**. Levels of ALP and IgM were also significantly lower in patients in the AMA-negative group as compared to the AMA-positive group. The degree of bile duct damage around the portal area was significantly milder in the AMA-positive group **[34]**.

In addition, Poupon et al reported that AMA titers decrease with treatment with UDCA **[35]**. We recently observed several cases of early-stage PBC in which AMA titers became negative after treatment with UDCA, indicating the presence of immunological complete remission after UDCA therapy **(unpublished data)**. Further prospective studies are needed to clarify the clinical significance of AMA levels over time with UDCA or bezafibrate therapy and to determine whether immunological complete remission can be achieved with treatment during an early stage of the disease, especially in AMA-positive subjects without any biochemical abnormalities.

III. Anti-nuclear antibodies (ANAs)

ANAs are detected by IIF in 30–50% of PBC patients **[5,6,8,9,36,37]**. ANAs detected by IIF using HEp-2 cells show various staining patterns, depending on the corresponding nuclear antigens **(Figure 1 and Table 1) [8,36,37]**. These antigens include components of (1) the NE pore complex (gp210 and p62), which correspond to a dotted NE or rim-like/membranous pattern **(Figure 1C)**; (2) lamin A, B, C, and lamin B receptor, which correspond to a smooth NE pattern **(Figure 1F)**; (3) sp100, PML proteins, sp140, and small ubiquitin-related modifiers (SUMOs), which correspond to a multiple nuclear dot (MND) pattern **(Figure 1D)**; and (4) centromere A, B, and C proteins, which correspond to a centromere (CENP) pattern **(Figure 1E) [8,37]**.

1: Anti-gp210 antibodies

Gp210 is an integral glycoprotein of the nuclear pore complex consisting of three main domains: a large glycosylated luminal domain, a single hydrophobic transmembrane segment, and a short cytoplasmic tail **[38,39]**. The main gp210 epitope recognized by anti-gp210 autoantibodies is a 15 amino acid stretch in the cytoplasmic, carboxyl-terminal domain of the protein, which is widely used for the detection of anti-gp210 antibodies in ELISAs **[39,40,41]**. Antibodies to gp210 are highly specific for PBC and are detected in 20–40% of PBC patients **[39,40,41]**. Itoh et al were the first to

report an association between anti-gp210 antibodies and disease severity in 1998 **[42]**. Subsequently, the clinical significance of anti-gp210 antibodies and antibodies to nuclear pore complexes in general has been described by several investigators **[43-48]**.

Nakamura et al made serial ELISA measurements of serum levels of antibodies to the terminal peptide of gp210-C 1863–1887 (PTSPNALPPARKASPPSGLWSPAYASH) in a large Japanese cohort of PBC patients **[41,48,49]**. Results obtained so far indicate that (1) serum titers of anti-gp210 antibodies change from negative to positive or vice versa depending on disease activity or stage progression; (2) anti-gp210 antibody positivity is associated histologically with more severe interface hepatitis, lobular inflammation, and ductular reaction; (3) persistently positive anti-gp210 antibodies are a strong risk factor for progression to end-stage hepatic failure (jaundice-type progression); and (4) the prognosis is most favorable in PBC patients who were initially seropositive for anti-gp210 antibodies but became negative after UDCA treatment. A good immunological response, as determined by anti-gp210 antibody levels, is the most significant protective factor associated with disease progression, in addition to other recently well described protective factors based on a good biochemical response to UDCA treatment **[50,51,52]**. A significant association between anti-gp210 antibody status and severity or progression of PBC has

been supported by other recent cohort studies in Japan and China **[22,53]**. Since UDCA therapy was introduced into clinical practice for PBC, the natural course of PBC has changed dramatically in patients who are positive and negative for anti-gp210 antibodies. However, anti-gp210 antibody positivity remains the strongest risk factor for progression to hepatic failure in PBC patients in the latest Japanese cohort study **[54]**. To better understand the clinical significance of anti-gp210 antibodies, further study is needed in association with other factors that may influence anti-gp210 antibody production such as HLA, as well as environmental and genetic factors in patients of various ethnicities **[49,55-57]**.

2: Anti-sp100 and anti-PML antibodies

The nuclear protein sp100 is a transcription factor that co-localizes with PML, another transcription coactivator **[37,38,39]**. Antibodies to sp100 and PML are detected in a dot-like distribution within the nucleus (MND pattern) with IIF **(Figure 1D) [8,37,58]**. Anti-sp100 and anti-PML antibodies are highly specific for PBC, with a prevalence of approximately 20–40% and 15–20%, respectively, in PBC patients **(Table1) [37,58-62]**. Antibodies to sp100 and PML typically co-exist in PBC; however, they do not cross-react **[58]**. In addition, approximately 74% of PBC patients with

urinary tract infections are positive for anti-sp100 antibodies, but only 4.8% of PBC patients do not have urinary tract infections **[61]**. These findings may support the hypothesis that some bacteria such as *Escherichia coli* are involved in the induction of autoantibodies in PBC **[61]**. Anti-sp100 or anti-PML antibody positivity is associated with disease severity and poor prognosis in European populations **[59,60,62]**. However, the significance of these antibodies remains to be determined in different ethnicities, including the Japanese population **[48]**.

3: Anti-centromere antibodies (ACAs)

Kinetochore is the protein structure on the centromere, which is composed of more than 100 distinct proteins. It is the site to which spindle fibers attach during cell division to pull sister chromatids apart **[9]**. ACAs, which were originally found in patients with systemic sclerosis (SSc), are found in a discrete speckled pattern of nuclear staining in HEp-2 cells **(Figure 1E) [9,37,63]**. Kinetochore proteins, including CENP-A (17 kDa), CENP-B (~80 kDa), and CENP-C (140 kDa), are the major autoantigens recognized by ACAs **[9]**. ACAs are detected in approximately 90% of patients with limited cutaneous systemic sclerosis (LcSS, formerly known as CREST syndrome: calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly,

and telangiectasia). ACAs are detected in up to 30% of patients with diffuse cutaneous SSc (dSSc) **[63,64]**. ACAs are also found in other autoimmune diseases including Sjogren's syndrome, systemic lupus erythematosus, rheumatoid arthritis, and PBC **[9]**.

Of note, ACA is detected in 10 to 30% of PBC patients without any apparent clinical manifestations of concomitant SSc **[5,6,37,48,49,53,65]**. The prevalence of ACAs in PBC patients is much higher than in patients with both PBC and SSC (1–2%) **[66]**, indicating that ACAs are present in the serum of PBC patients without evidence of co-existing SSc. In addition, ACAs are significantly associated with the pathogenesis of PBC along with other PBC-specific autoantibodies such as AMA, anti-gp210, and anti-sp100.

One clinically relevant question is whether ACAs are a pre-clinical marker of future SSc in patients with PBC alone, or whether ACA-positive PBC patients have a subclinical form of the disease. To address this, ACA-positive PBC patients have been studied with regards to disease severity, prognosis, and clinical phenotype **[48,49]**. The results from one Japanese cohort so far indicate that (1) ACSs, in contrast to anti-gp210 antibodies, are detected before the onset of PBC, and ACA titers are stable (i.e., they do not change from positive to negative or vice versa over time); (2) PBC patients who are positive for ACAs are at high risk for progression to

cirrhosis and portal hypertension, but not to persistent jaundice and hepatic failure; (3) ACA positivity is histologically associated with more severe ductular reaction **[48,49]**. These findings are supported by additional studies **[46, 53]**, but there are also reports indicating that ACA-positive PBC patients do not differ from ACA-negative PBC patients **[65,66]**. Since ACAs and anti-gp210 antibodies tend to be mutually exclusive, the significance of ACAs in the progression of PBC must be carefully evaluated in association with other risk factors including HLA and the presence of anti-gp210 antibodies **[49]**. SSc patients who are positive for ACAs invariably have Raynaud's phenomenon. ACAs are also associated with an elevated risk of pulmonary hypertension but not pulmonary fibrosis, indicating that ACAs can be a marker for differentiating between clinical phenotypes of SSc **[63]**. The mechanism of ACA production and its clinical significance as a marker for differentiating between clinical phenotypes in PBC remain important issues to be addressed.

4: Other ANAs detected in PBC

Autoantibodies to SUMOs and sp140, which appear in a MND pattern, and autoantibodies to p62 and lamin B receptor, which appear in a NE, are detected in 2–9%, 15%, 10–30%, and 2–9 %, respectively, of PBC patients in a disease-specific

manner **(Table 1) [37,38,39,45,68,69]**. However, the clinical significance of these autoantibodies remains to be determined in patients of different ethnicities.

IV. Three types of PBC progression

Three major forms of PBC were identified by Poupon et al **[2]**: (1) the typical or classical form, represented by a slow, progressive loss of small bile ducts and parallel increase in liver fibrosis, leading to biliary cirrhosis over a period of 10 to 20 years; (2) fluctuating or persistent presence of AIH features associated with early development of liver fibrosis and liver failure, seen in 10–20% of patients; and (3) the so-called premature ductopenic variant seen in 5–10% of patients, represented by a very rapid onset of ductopenia and severe icteric cholestasis with progression to cirrhosis and liver failure in less than 5 years. Within the typical or classical variant of PBC described above, we found that there are three types of clinical evolution in a large cohort study in Japan: (1) minimum to very slow progression over time, (2) relatively slow progression to cirrhosis or portal hypertension without the development of persistent jaundice or hepatic failure (portal hypertension-type progression), and (3) relatively rapid progression to jaundice and hepatic failure (jaundice-type progression) **(Figure 2) [10,11,48,49]**. Based on this classification, anti-gp210 antibody positivity is the strong

risk factor for jaundice-type progression, while ACA positivity is a risk factor for portal hypertension-type progression **(Figure 2) [10,11,48,49]**. Since the natural course of PBC is being altered by treatment with UDCA, the clinical significance of these PBC-specific autoantibodies awaits re-evaluation in prospective studies in various ethnicities.

V. Conclusion

It is of note that the autoimmune repertoire as represented by autoantibody status, which is established during the early stages of the disease process, can influence the clinical phenotype and long-term prognosis of PBC. In addition, this autoimmune status can be normalized by treatment with UDCA, given that anti-gp210 antibodies can become negative with UCDA therapy; even AMAs can become negative with UDCA therapy in some patients in an early phase of the disease **[41,48,49,54]**. To understand the mechanisms of autoantibody production (i.e. AMAs, anti-gp210 antibodies, anti-sp100 antibodies, and ACAs) and its clinical significance, further studies are needed in association with environmental and genetic factors that might influence the production of these autoantibodies in PBC.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

This work was supported by Grants-in-Aid for Scientific Research from the Japan Society for Promotion of Science (#20590800, #23591006), a Grant-in-Aid for Clinical Research from the National Hospital Organization (NHO), and by the Research Program of Intractable Disease provided by the Ministry of Health, Labour and Welfare of Japan to Minoru Nakamura.

Abbreviations

ACAs	anti-centromere antibodies
AMAs	anti-mitochondrial antibodies
ANAs	anti-nuclear antibodies
BCOADC-E2	E2 component of branched chain 2-oxoacid dehydrogenase complex
CENP	centromere protein
dSSc	diffuse cutaneous systemic sclerosis
IIF	indirect immunofluorescence

MND	multiple nuclear dot
LcSSc	limited cutaneous systemic sclerosis
NE	nuclear envelope
NPC	nuclear pore complex
Nup	nucleoporin
OGDC-E2	E2 component of the 2-oxo-glutarate dehydrogenase complex E2
PBC	primary biliary cirrhosis
PDC-E2	E2 component of the pyruvate dehydrogenase complex
PML	promyelocytic leukemia protein
SUMO	small ubiquitin-related modifier
UDCA	ursodeoxycholic acid

V: References

1 Kaplan MM, Gershwin ME. Primary biliary cirrhosis. N EnglJ Med 2005;353:1261-73

2 Poupon R. Primary biliary cirrhosis: A 2010 update. J Hepatol2010; 52:745-758

3 Lindor KD, Gershwin ME, Poupon R, et al.Hepatology2009;50:291-308

4 Beuers U, Boberg KM, Chapman RW, et al. EASL Clinical Practice Guide-lines: management of cholestaticliver diseases. J Hepatol 2009; 51:237-67

5 Czaja AJ. Autoantibodies as prognostic markers in autoimmune liverdisease. Dig Dis Sci 2010; 55: 2144-2161

6 Hu C-H, Zhang F-C, Li Y-Z, et al. Primary biliary cirrhosis: What do autoantibodies tell us? World J Gastroenterol 2010; 16:3616-3629

7 Leung PSC, Coppel RL, Ansari A, et al. Antimitochondrial antibodies in primary biliary cirrhosis. Semin Liver Dis 1997; 17:61-69

8 Invernizzi P, Selmi C, Ranftler C, Podda M, Wesierska-Gadek J. Antinuclear antibodies in primary biliary cirrhosis. Semin Liver Dis 2005; 25: 298-310

9 Liberal R, Grant CR, Sakkas L, et al. Diagnostic and clinical significance of anti-centromere antibodies in primary biliary cirrhosis. Clin Res Hepatol Gastroenterol 2013; 37:572-585

10 Nakamura M, Komori A, Ito M, et al. Predictive role of anti-gp210 and anticentromere antibodies in long-term outcome of primary biliary cirrhosis. Hepatol Res 2007; **37** Suppl 3: S412-S419

11 Ishibashi H, Komori A, Shimoda S, et al. Risk factors and prediction of long-term

outcome in primary biliary cirrhosis. Internal Medicine 2010; 50:1-110

12 Hirschfield GM, Gershwin E. Primary biliary cirrhosos: One disease with many faces. Isr Med Assoc J 2011; 13:55-9

13 Gershwin ME, Mackay IR, Sturgess A, Coppel RL. Identification and specificity of a cDNA encoding the 70 kd mitochondrial antigen recognized in primary biliary cirrhosis. J Immunol 1987; 138:3525-31

14 Yeaman SJ, Fussey SP, Danner DJ, et al. Primary biliary cirrhosis: identification of two major M2 mitochondrial autoantigens. Lancet 1988; 1:1067-70

15 Fussey SPM, Guest JR, James OFW, et al. Identification and analysis of the major M2 autoantigens in primary biliary cirrhosis. Proc Natl Acad Sci USA 1988; 85:8654-8658

16 Gershwin ME, Rowley M, Davis PA, et al. Molecular biology of the 2-oxo-acid dehydrogenase complexes and anti-mitochondrial antibodies. Prog Liver Dis 1992; 10: 47-61

17 Moteki S, Leung PSC, Coppel RL, et al. Use of a designer triple expression hybrid clone for three different lipoyl domains for the detection of antimitochondrial autoantibodies. Hepatology 1996; 24:97-103

18 Oertelt S, Rieger R, Selmi C, et al. A sensitive bead assay for antimitochondrial

antibodies: chipping away at AMA-negative primary biliary cirrhosis. Hepatology 2007; 45:659-65

19 Dahnrich C, Pares A, Caballeria L, et al. New ELISA for detecting primary biliary cirrhosis-specific antimitochondrial antibodies. Clinical Chemistry 2009; 55:978-985

20 Liu H, Norman GL, Shums Z, et al. PBC Screen: An IgG/IgA dual isotype ELISA detecting multiple mitochondrial and nuclear autoantibodies specific for primary biliary cirrhosis. J Autoimmun 2010; 436-442

21 Bizzaro N, Covini G, Rosina F, et al. Overcoming a "probable" diagnosis in antimitochondrial antibody negative primary biliary cirrhosis: study of 100 sera and review of the literature. Clin Rev Allerg Immunol 2012; 42:288-297

22 Saito H, Takahashi A, Abe K, et al. Autoantibodies by line immunoassay in patients with primary biliary cirrhosis. Fukushima J Med Sci 2012; 58:107-116

23 Lazaridis KN, Juran BD, Boe GM, et al. Increased prevalence of antimitochondrial antibodies in first-degree relatives of patients with primary biliary cirrhosis. Hepatology 2007; 46: 785-792

24 Mitchison HC, BassendineMF, Hendrick A, et al. Positive antimitochondrial antibody but normal alkaline phosphatase: is this primary biliary cirrhosis?

Hepatology1986; 6: 1279-1284

25 Metcalf JV, Mitchison HC, Palmer JM, et al. Natural history of early primary biliary cirrhosis. Lancet 1996; 348:1399-402.

26 Mattalia A, Quaranta S, Leung PS, et al. Characterization of anti- mitochondrial antibodies in health adults. Hepatology1998; 27: 656-661

27 Benson GD, Kikuchi K, Miyakawa H, et al. Serial analysis of antimitochondrial antibody in patients with primary biliary cirrhosis. Clin Dev Immunol 2004; 11: 129-133

28 Van Norstrand MD, Malinchoc M, Lindor KD, et al. Quantitative measurements of autoantibodies to recombinant mitochondrial antigens in patients with primary biliary cirrhosis: relationship of levels of autoantibodies to disease progression. Hepatology 1997; 25:6-11

29 Nakanuma Y, Harada K, Kaji K, et al. Clinicopathological study of primary biliary cirrhosis negative for antimitochondrial antibodies. Liver 1997; 17:281-287

30 Invernizzi P, Crosignani A, Battezzati PM, et al. Comparison of the clinical features and clinical course of antimitochondrial antibody-positive and -negative parimary biliary cirrhosis. Hepatology 1997; 25:1090-1095

31 Liu B, Shi XH, Zhang FC, Zhang W, Gao LX. Antimitochondrial

antibody-negative primary cirrhosis: a subset of primary biliary cirrhosis. Liver Int 2008; 28:233-9

32 Hirschfield GM, Heathcote EJ. Antimitochondrial antibody-negative primary biliary cirrhosis. Clin Liver Dis 2008; 12:323-331

33 Sakaguchi F, Mori M, Zeniya M, et al. Antimitochondrial antibody negative primary biliary cirrhosis in Japan; utilization of clinical data when patients applied to receive public finantial aid. J Epidemiol 2006; 16:30-34

34 Jin Q, Moritoki Y, Lleo A, et al. Comparative analysis of portal cell infiltrates in AMA positive versus AMA negative PBC. Hepatology 2012; 55:1495-1506

35 Poupon RE, Balkau B, Eschwège E, Poupon R. A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. UDCA-PBC Study Group. N Engl J Med 1991; 324: 1548-1554

36 Worman HJ, Courvalin J-C. Antinuclear antibodies specific for primary biliary cirrhosis. Autoimmun Rev 2003; 2:211-21

37 Granito A, Muratori P, Quarneti C. Antinuclear antibodies as ancillary markers in primary biliary cirrhosis. Expert Rev Mol Diagn 2012; 12:65-74

38 Worman HJ, Courvalin J-C. Autoantibodies against nuclear envelope proteins in

liver disease. Hepatology 1991; 14:1269-79

39 Courvalin J-C, Worman HJ. Nuclear envelope protein autoantibodies in primary biliary cirrhosis. Sem Liver Dis 1997; 17:79-90

40 Bandin O, Courvalin JC, Poupon R, et al. Specificity and sensitivity of gp210 autoantibodies detected using enzyme-linked immunosorbent assay and a synthetic polypeptide in the diagnosis of primary biliary cirrhosis. Hepatology 1996; 23:1020-1024

41 Nakamura M, Shimizu-Yoshida Y, Takii Y, et al. Antibody titer to gp210-C terminal peptide as a clinical parameter for monitoring primary biliary cirrhosis. J Hepatol 2005; 42: 386-392

42 Itoh S, Ichida T, Yoshida T, et al. Autoantibodies against a 210kDa glycoprotein of the nuclear pore complex as a prognostic marker in patients with primary biliary cirrhosis. J Gastroenterol Hepatol 1998; 13: 257-265

43 Invernizzi P, Podda M, Battezzati PM, et al. Autoantibodies against nuclear pore complexes are associated with more active and severe liver disease in primary biliary cirrhosis. J Hepatol 2001; 34:366-372

44 Muratori P, Muratori L, Ferrari R, et al. Characterization and clinical impact of antinuclear antibodies in primary biliary cirrhosis. Am J Gastroenterol 2003; 98:

431-437

45 Miyachi K, Hankins RW, Matsushima H, et al. Profile and clinical significance of anti-nuclear envelope antibodies found in patients with primary biliary cirrhosis: a multicenter study. J Autoimmun 2003; 20: 247-254

46 Yang W-H, Yu JH, Nakajima A, et al. Do antinuclear antibodies in primary biliary cirrhosis patients identify increased risk for liver failure? Clin Gastroenterol Hepatol 2004; 2:1116-1122

47 Wesierska-Gadek J, Penner E, Battezzati PM, et al. Correlation of initial autoantibody profile and clinical outcome in primary biliary cirrhosis. Hepatology 2006; 43: 1135-1144, 2006.

48 Nakamura M, Kondo H, Mori T, et al. Anti-gp210 and anticentromere antibodies are different risk factors for the progression of primary biliary cirrhosis. Hepatology 2007; 45: 118-127

49 Nakamura M, Yasunami M, Kondo H, et al. Analysis of HLA-DRB1 polymorphisms in Japanese patients with primary biliary cirrhosis (PBC): The HLA-DRB1 polymorphism determines the relative risk of antinuclear antibodies for disease progression in PBC. Hepatol Res 2010; 40: 494-504

50 Pares A, Caballeria L, Rodes J. Excellent long-term survival in patients with

primary biliary cirrhosis and biochemical response to ursodeoxycholic acid. Gastroenterolgy 2006; 130:715-20

51 Kumagi T, Guindi M, Fischer SE, et al. Baseline ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis. Am J Gastroenterol 2010; 105:2186-94

52 Corpechot C, Chazouilleres O, Poupon R. Early primary biliary cirrhosis: biochemical response to treatment and prediction of long-term outcome. J Hepatol 2011; 55:1361-7

53 Gao L, Tian X, Liu B, Zhang F. The value of antinuclear antibodies in primary biliary cirrhosis. Clin Exp Med 2008;8:9-15.

54 Nakamura M, Kondo H, Tanaka A, et al. Autoantibody status and histological variables influence biochemical response to treatment and long-term outcomes in Japanese patients with primary biliary cirrhosis. Submitted for publication

55 Nakamura M, Nishida N, Kawashima M, et al. Genome-wide association study identifies TNFSF15 and POU2AF1 as susceptibility loci for primary biliary cirrhosis in the Japanese population. Am J Hum Genet 2012; 91:721-8.

56 Invernizzi P, Ransom M, Raychaudhuri S, et al. Classical HLA-DRB1 and DPB1 allele account for HLA association with primary biliary cirrhosis. Genes Immun.

2012; 13:461-468

57 Kar SP, Seldin MF, Chen W, et al. Pathway-based analysis of primary biliary cirrhosis genome-wide association studies. Genes Immun 2013; 14:179-86

58 Szostecki C, Guldner HH, Will H. Autoantibodies against "nuclear dots" in primary biliary cirrhosis. Semin Liver Dis 1997; 17: 71-78

59 Züchner D, Sternsdorf T, Szostecki C, et al. Prevalence, kinetics, and therapeutic modulation of autoantibodies against Sp100 and promyelocytic leukemia protein in a large cohort of patients with primary biliary cirrhosis. Hepatology1997; 26: 1123-1130

60 Rigopoulou EI, Davies ET, Pares A, et al. Prevalence and clinical significance of isotype specific antinuclear antibodies in primary biliary cirrhosis. Gut 2005; 54:528-532

61 Bogdanos DP, Baum H, Butler P, et al. Association between the primary biliary cirrhosis specific anti-sp100 antibodies and recurrent urinary tract infection. Dig Liver Dis 2003; 35:801-805

62 Mytilinaiou MG, Meyer W, Scheper T, et al. Diagnostic and clinical utility of antibodies against the nuclear body promyelocytic leukemia and sp100 antigens in patients with primary biliary cirrhosis. Clin Chim Acta 2012;

413:1211-6

63 Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. N Engl J Med 2009; 3 60:1989-2003.

64 Reveille JD, Solomon DH. Evidence-based guidelines for the use of immunologic tests: anticentromere, Scl-70, and nucleolar antibodies. Arthritis Rheum 2003; 49:399-412.

65 Parveen S, Morshed SA, Nishioka M. High prevalence of antibodies to recombinant CENP-B in primary biliary cirrhosis: nuclear immunofluorescence patterns and ELISA reactivities. J Gastroenterol Hepatol 1995; 10:438-45

66 Rigamonti C, Shand LM, Feudjo M, et al. Clinical features and prognosis of primary biliary cirrhosis associated with systemic sclerosis. Gut 2006; 55:388-94

67 Shi T-Y, Zhang L-N, Chen H, et al. Risk factors for hepatic decompensation in patients with primary biliary cirrhosis. World J Gasroenterol 2013; 19:1111-1118

68 Granito A, Yang W-H, Muratori L, et al. PML nuclear body component Sp140 is a novel autoantigen in primary biliary cirrhosis. Am J Gastroenterol 2010; 105:125-131

69 Wesierska-Gadek J, Klima A, Ranftler C, et al. Characterization of the antibodies to p62 nucleoporin in primary biliary cirrhosis using human recombinant antigen. J

cell Biochem 2008; 27-37

Figure Legends

Figure 1 AMAs and ANAs detected in PBC.

A: AMAs detected by IIF using sections of rat kidney and stomach. Positive immunofluorescence is observed in the mitochondria-rich kidney (left) and the parietal cells of the stomach (right), but not in the smooth muscle of the stomach (middle).

B: AMA detected by IIF in HEp-2 cells. The mitochondria in the cytoplasm are positively stained.

C: Nuclear envelope (NE) pore complexes (gp210, p62) are positively stained in a NE pattern.

D: Nuclear proteins (sp100, PML, sp140, SUMO) are positively stained in a multiple nuclear dot (MND) pattern.

E: Centromere proteins (centromere protein A, B, C) are positively stained in a discrete speckled pattern (centromere pattern).

F: Nuclear lamina (lamin A, B, C, and lamin B receptor) are positively stained in a smooth rim-like pattern.

Figure 2　Three types of PBC progression.

Anti-gp210 antibody positivity is a risk factor for hepatic failure-type progression, while anti-centromere antibody positivity is a risk factor for portal hypertension-type progression in Japanese cohort studies **[10,11,41,48,49]**.

Figure 1

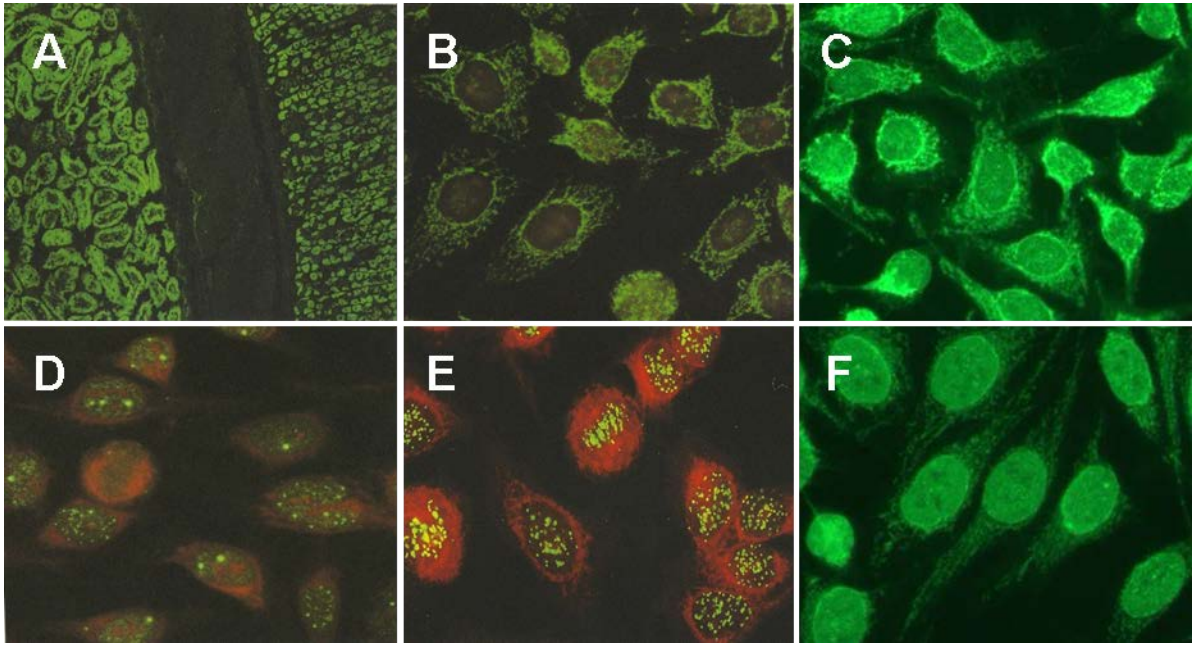

Figure 2

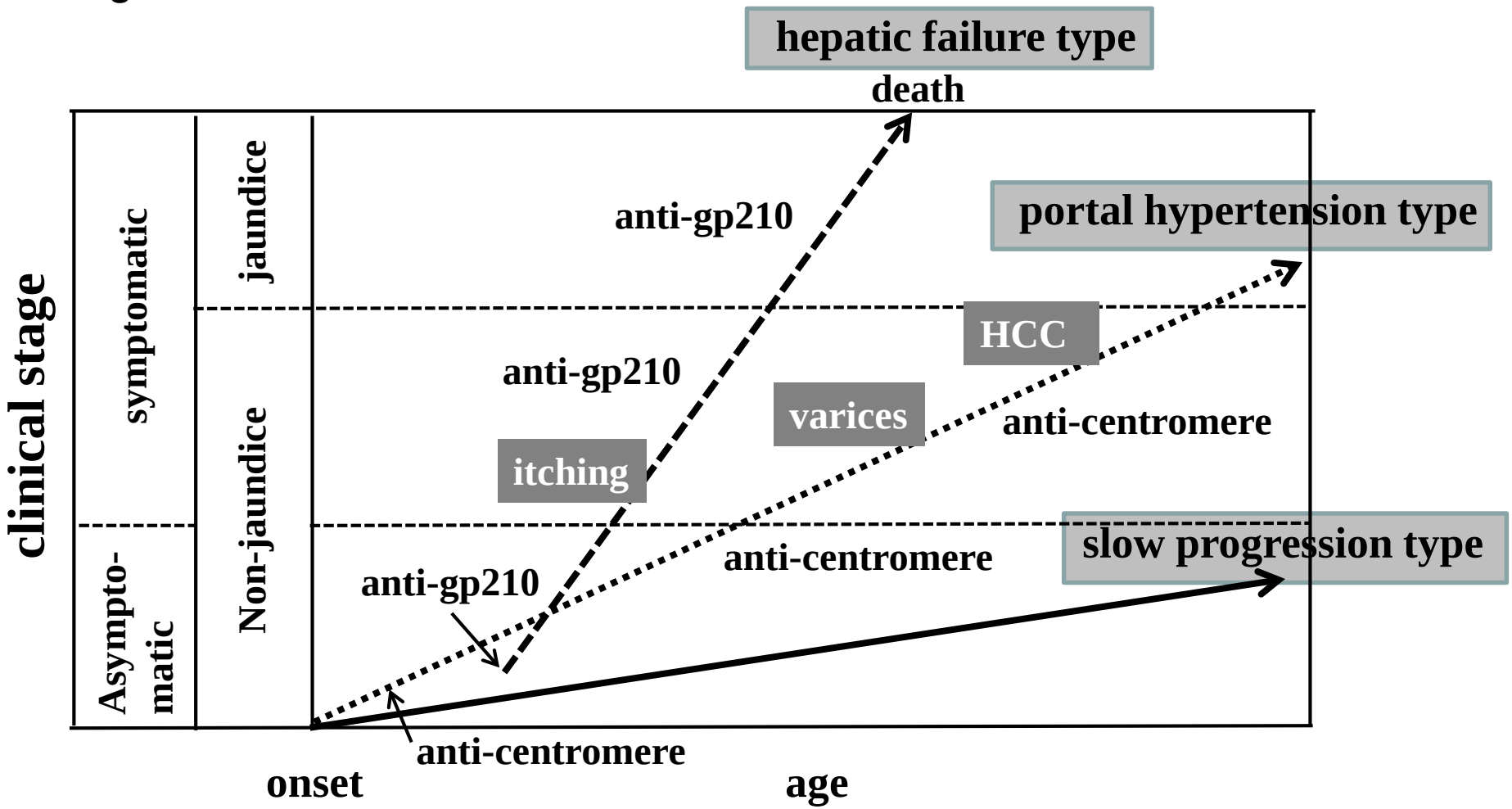
hepatic failure type
death
portal hypertension type
slow progression type
clinical stage
symptomatic
Asympto-matic
jaundice
Non-jaundice
anti-gp210
anti-gp210
anti-gp210
HCC
varices
itching
anti-centromere
anti-centromere
anti-centromere
onset
age

Table 1. Clinical significance of autoantibodies detected in PBC

Autoantibody	IIF pattern	Sensitivity for PBC	Specificity for PBC	Other diseases	Clinical significance
Anti-mitochondrial antibodies (AMAs)	MIT	90–95 %	high	none	Dx of PBC No difference in clinical features between AMA-positive and AMA-negative patients
Anti-PDC-E2 (74 kDa)		80–90 %			
Anti-PDC-E3BP (50 kDa)		10%			
Anti-PDC-E1a (41 kDa)		5–25 %			
Anti-OGDC-E2 (48 kDa)		20–60 %			
Anti-BCOADC-E2 (52 kDa)		50–80 %			
Anti-nuclear antibodies (ANAs)		40–50 %	Depends on target antigen		
Anti-gp210	NE	10–40 %	very high	none	Dx of AMA-negative PBC, hepatic failure-type progression
Anti-p62		10–30 %	high	SjS	Dx of AMA-negative PBC, more severe disease
Anti-lamin B receptor		2–9 %	high	none	Dx of AMA-negative PBC, association with clinical features ?
Anti-sp100	MND	20–40 %	high		Dx of AMA-negative PBC, faster progression
Anti-PML		15–20 %	high		
Anti-sp140		15%	high		Dx of AMA-negative PBC, association with clinical features ?
Anti-SUMO-1,2		2–6 %	high		
Anti-centromere A, B, C	CENP	10–30 %	not high	SSc	Portal hypertension-type progression

MIT: mitochondria pattern, NE: nuclear envelope (rim-like/membranous), MND: multiple nuclear dot, CENP: centromere pattern, Dx: diagnosis, IIF: indirect immunofluorescence with HEp-2 cells

SjS: Sjogren's syndrome, SSc: systemic sclerosis