INTERNAL MEDICINE

□ REVIEW ARTICLE □

Risk Factors and Prediction of Long-term Outcome in Primary Biliary Cirrhosis

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Abstract

The natural history of the disease varies greatly among individual patients with primary biliary cirrhosis (PBC). Some patients live long without any symptoms while other patients present jaundice and develop hepatic failure in early phases of the disease. Previous studies showed that the natural course of PBC is altered by the use of ursodeoxy cholic acid (UDCA). In this review we discuss variation in the natural course of the disease and it's alteration by UDCA, and risk factors that predict disease progression. Based on clinical observations, there are three types of clinical evolution in PBC: 1) minimal to slow progression over several years; 2) rapid progression to jaundice and hepatic failure, and 3) progression to portal hypertension without developing deep jaundice. Notably, based on our analyses accelerated progression to jaundice and liver failure are reflected by a sustained serologic presence of anti-gp210 antibodies whereas patients with portal hypertension in the absence of jaundice have anti-centromere autoantibodies. These observations highlight the clinical importance of antinuclear antibody analysis in patients with PBC.

Key words: primary biliary cirrhosis, natural history, prognosis, autoantibody, gp210, centromere

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Introduction

Primary biliary cirrhosis (PBC) is a chronic autoimmune cholestatic liver disorder which has the potential to progress to cirrhosis and eventually hepatic failure (1). The disease progression of patients with PBC in fact varies significantly among patients, including a relatively long asymptomatic phase in some patients while some have a more fulminate onset that progress to severe disease. Recent advances in the diagnosis of PBC, especially the discovery of anti-mitochondrial antibody (AMA), have led to most patients being recognized in the earlier, asymptomatic stage, and furthermore, many respond well to medical therapy using ursodeoxycholic acid (UDCA) such that their survival becomes significantly prolonged. Thus, currently the natural course of PBC is different from that in the pre-UDCA era. In the absence of UDCA therapy, PBC patients with early disease had a shortened survival compared to a healthy population regardless of symptoms (2, 3). However, the survival for UDCA-treated patients with early PBC is now significantly prolonged to a level comparable to that of the general population (4). Presently, however, there are no prognostic markers that identify those patients in the asymptomatic stage that will develop progressive disease from those who will remain symptom-free. We recently found that the persistent presence of anti-gp210 antibody is a biomarker for poor progression of patients with PBC (5). Thus, determining the presence of anti-gp210 in PBC patients is pivotal in predicting the prognosis of the disease, which is vital in the management of PBC patients, both for planning surgical therapy and for counseling patients (3).

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Natural History

Characteristic autoantibodies in PBC, anti-mitochondrial antibodies

The serological hallmark of PBC is the presence of AMA, a highly disease-specific autoantibody found in 90-95% of patients and less than 1% of normal controls (6). Since its discovery, the detection of AMA has become a major diagnostic criterion for PBC. The actual autoantigens of the anti-mitochondrial response have been revealed to be the E2-components of the 2-oxo-acid dehydrogenase family of enzyme complexes (2-OADC) including pyruvate dehydrogenase complex (PDC-E2), branched chain 2-oxoacid dehydrogenase complex (BCOADC-E2), and 2-oxo-glutarate dehydrogenase complex (OGDC-E2). These enzymes are located in the inner mitochondrial membrane and catalyze the oxidative decarboxylation of keto-acid substrates (7).

Early PBC with detectable AMA but no abnormal liver function tests

The presence of AMA could be the first expression of PBC and could be found in asymptomatic stage or the absence of abnormal liver enzymes, such as alkaline phosphates and γ-glutamyl transpeptidase (8). To elucidate the frequency and antigen specificity of AMAs in the asymptomatic population and to identify patients with early PBC, Mattalia et al investigated the prevalence of AMA in a healthy population, and found that approximately 0.5% of the population is positive for AMA (8). Importantly, the pattern of reactivity to PDC-E2 in non-PBC individuals differed from that found in PBC patients in most of the AMA positive sera. After a variable follow-up period (8-14 months), the reactivity of sera from 8 of 9 had a wider AMA pattern than before. This study demonstrated that AMA as a "natural" autoantibody is different from a "pathological" autoantibody in PBC patients.

Mitchison et al (9) studied a group of 29 AMA positive patients without symptoms but with normal liver function test to establish whether patients with AMA have very early PBC and to follow the disease progression. Overall, 76% of AMA positive individuals developed symptoms of PBC and 83% had liver function tests persistently showing cholestasis (10). This study confirmed that individuals, who are repeatedly AMA positive with no symptoms and normal liver enzyme levels, tend to have very early PBC.

Progression from asymptomatic to symptomatic PBC in the pre-UDCA era

Clinically, PBC patients can progress from an asymptomatic stage to a symptomatic stage with symptoms attributable to liver damage, such as itching, jaundice, esophageal varices, edema, ascites and/or encephalopathy (3). Some patients have a very long latent or asymptomatic stage before they become symptomatic. The prognosis of asymptomatic PBC patients is very good for as long as they remain in the asymptomatic stage. The 10-year survival of asymptomatic patients ranged from 50 to 70%; whereas the median duration of survival for symptomatic patients ranged from 5 to 8 years from the onset of symptoms (2, 11). However, it must be noted that a study of 279 patients from the United States (11) demonstrated that the overall survival of those asymptomatic patients was shorter than that predicted for an age- and gender-matched control population ($p<0.0001$). Nevertheless, the development of symptoms could not be predicted by either serum biochemical tests or hepatic histology in these studies. These findings suggest that despite the less severe clinical signs in asymptomatic PBC than in symptomatic PBC, the prognosis is not associated with the existence of symptoms in PBC.

Progression of fibrosis and development of complications related to cirrhosis

Histologically, in PBC there is a characteristic chronic inflammatory infiltrate that surrounds and invades biliary ductules in the portal areas of the liver, called chronic non-suppurative destructive cholangitis, together with granuloma formation (12). PBC is classified into four histological stages: stage one, inflammation and/or abnormal connective tissue confined to the portal areas; stage two, inflammation and/or fibrosis confined to portal and periportal areas; stage three, bridging fibrosis and stage four; cirrhosis (12).

The histological stage is predictive of survival (12). In one study, cirrhosis was developed within four years in 31% and 50% of those patients who were initially diagnosed as stage I and stage II, respectively (13). Only 20% of the pre-cirrhotic patients showed histological stability and a sustained histological regression was rare (2%). A significant proportion of patients were also shown to develop liver failure, required transplantation or died; 26% of patients developed liver failure by 10 years after diagnosis (3).

The group of patients with cirrhosis has the worst outcome and they are at risk for variceal bleeding and hepatocellular carcinoma (HCC) (14, 15). In a study of 667 PBC patients followed over a 20-year period, HCC developed in 5.9% of the 273 patients in the III or IV stage of the disease (16) while HCC was not seen in any of the 394 patients in stage I or II. Among the study population, the incidence of HCC was increased in male, those of older age, or with a history of blood transfusions (14). Similar results were demonstrated in Japanese PBC patients (15). Several studies have addressed the risk of specific malignancies in PBC. Nijhawan et al (17) showed that there was a markedly increased risk for development of hepatobiliary malignancies. These studies suggest that histological changes provide prognostic information and they would be helpful not only in assessing the prognosis and therapeutic benefit but also in clinical design. Clearly, more studies need to be done to confirm the risk assessment of hepatobiliary malignancies in PBC.

Alteration of natural history by ursodeoxycholic acid

Though in the past there had been controversy on their efficacy, the consensus is that UDCA is the appropriate first-line drug for PBC worldwide. Several studies including randomized trials have shown that UDCA not only improves biochemical indices but also delays histologic progression and improves survival without transplantation (18-25). An early study demonstrated that the rate of histological progression to cirrhosis was significantly lower in the UDCA-treated group than in controls (18). A recent report on 192 patients with long-term follow-up showed that 61% of the patients responded to treatment. The long-term survival rate for UDCA-treated patients is similar to the matched control population, which supports the significant effects of UDCA treatment in PBC. Also, PBC patients who fail to show a biochemical response to UDCA progress histologically during extending follow-up (26). The effect of UDCA is more evident in early stages of the disease. The survival rate of patients in stage I and II was similar to that in the control population, whereas in treated patients ascertained at late histologic stages, the probability of death or liver transplantation remained significantly increased (23). A report from Japan shows that UDCA administration was a useful prognostic indicator among asymptomatic patients (20). These findings have important implications for future UDCA treatment strategies from asymptomatic stage in PBC. However, there is a continued need for new therapeutic options in patients who do not respond to UDCA or patients with advanced disease (24, 25).

Risk Factors Influence on the Prognosis

Recent analyses of familial and geographic clustering of PBC patients and environmental risk factors support the hypothesis that development and progression of the disease hinge on a complex interplay between genetic and environmental risk factors.

Genetic predisposition

Although the inheritance of PBC from patient to child is not generally evident, a strong genetic predisposition is indicated by the higher incidence of the disease in direct relatives and particularly among siblings and monozygotic twins (27, 28). Several individual genetic loci have been studied. The major histocompatibility complex (MHC) associations vary among studies. Polymorphisms of genes involved in immunity and tolerance (*CTLA4* and *TNF*α) were associated with outcome and response to UDCA therapy (29). Clearly, more studies are needed in order to understand the relevance of these observations for PBC treatments or in understanding the natural history of PBC.

Genetic loci conferring a risk for PBC-genome wide association analysis

Hirschfield et al (30) recently reported significant associations between PBC and common genetic variants at loci for HLA class II, IL12A, and IL12RB2 loci utilizing a genome wide association analysis. They found significant associations between PBC and 13 loci across the HLA class II region. Within these loci, the HLA-DQB1 locus (encoding MHC class II, DQ β chain 1) had the strongest association with PBC. PBC was also shown to be significantly associated with two single-nucleotide polymorphisms (SNPs) at the IL12A locus (encoding interleukin-12α), rs6441286 and rs574808, and one SNP at the IL12RB2 locus (encoding interleukin-12 receptor β2), rs3790567. Further study by Hirschfield et al via combined analysis of the genome-wide association and replication datasets identified *IRF5-TNPO3* (combined $p=8.66\times10^{-13}$), 17q12-21 (combined $p=3.50\times10^{-13}$) and *MMEL1* (combined $p=3.15\times10^{-8}$) as new PBC susceptibility loci. Fine-mapping studies showed that a single variant accounts for the *IRF5-TNPO3* association (31). A genome-wide association screen for PBC risk alleles was also performed in an Italian cohort. The results from the Italian cohort replicated IL12A and IL12RB associations, and a combined meta-analysis using a Canadian dataset identified newly associated loci at SPIB ($p=7.9\times10^{-11}$, odds ratio (OR)=1.46), IRF5-TNPO3 ($p=2.8\times10^{-10}$, OR=1.63) and $17q12^{-21}$ ($p=1.7\times10^{-10}$, OR=1.38) (32).

Environmental factors

Environmental triggers appear to be crucial to disrupt the pre-existing weakness of immune tolerance in genetically predisposed individuals leading to the emergence of clinical disease after a long latency. Epidemiologic studies have suggested the association of PBC with infections, especially of the urinary tract, reproductive hormone replacement, use of nail polish and cosmetics, past cigarette smoking and living near toxic waste sites (33-36). Also, the studies demonstrating environmental xenobiotics inducing PBC-like disease in mice strongly suggest the significance of environmental factors in the pathological progression of PBC (36). McNally et al recently found a role of transient environmental factors, especially infectious factor, in the cause of PBC by examining population-based data and analyzing space-time clustering (37).

Autoantibodies

Several types of autoantibody occur in the sera of PBC patients (38). Virtually all PBC patients have AMA, but, interestingly approximately 30 to 50% of PBC patients also have antinuclear antibodies (ANAs). Yang et al (39) demonstrated that ANAs in general, but particularly anti-centromere antibodies are associated with the risk for hepatic failure in PBC, whereas other reports did not show any such effect (40). Although the significance and predictive value of ANAs have been confirmed in other autoimmune diseases such as type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosus and inflammatory bowel disease, the significance of ANAs for PBC remains unclear (41-43).

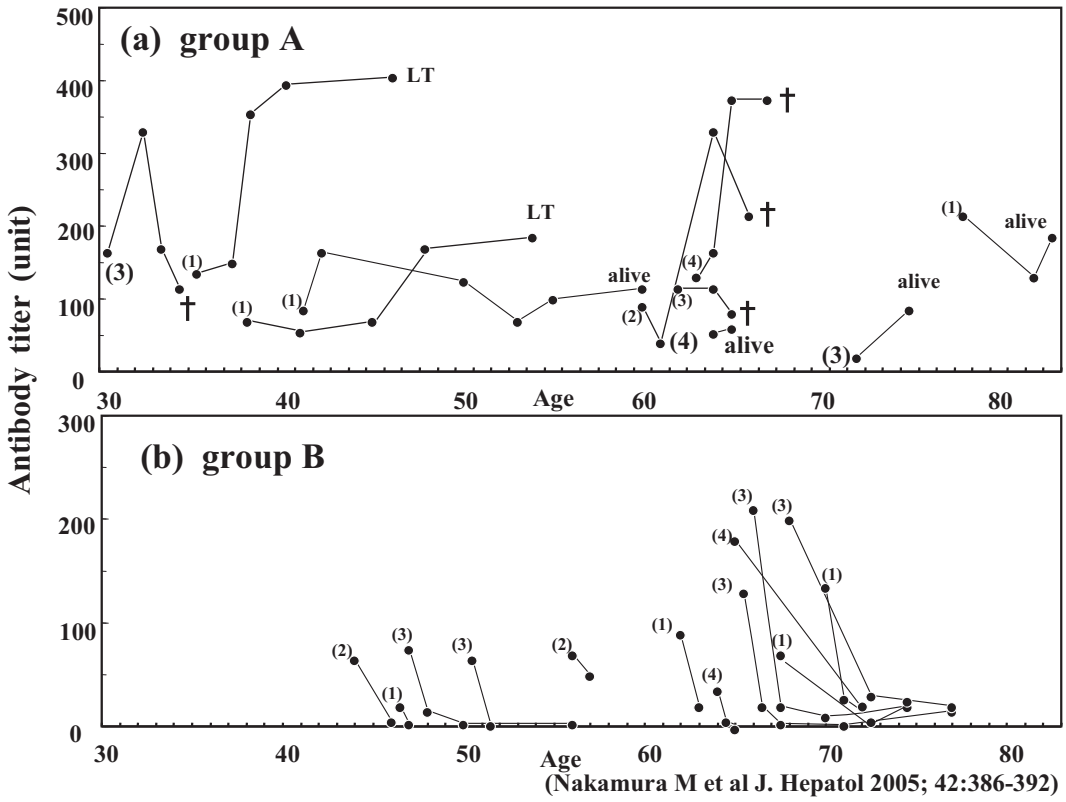


Figure 1. **Change of the antibody titer and the prognosis of patients.**

Anti-gp210 and anti-centromere autoantibodies

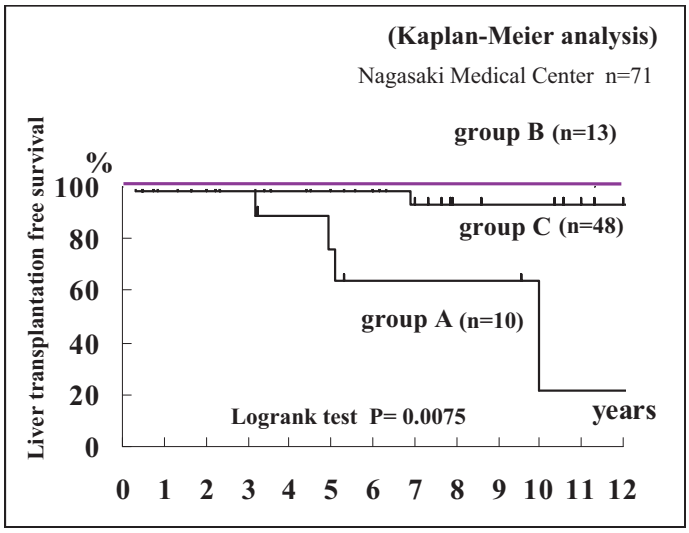
The association between the presence of anti-nuclear pore complexes as detected by anti-gp210 antibodies and the outcome of PBC was first reported by Itoh et al (44) and confirmed by Invernizzi et al (45), Muratori et al (46) and Miyachi et al (47). Wesierska-Gadek et al (48) recently reported the significance of anti-gp210 antibodies in the long-term outcome of PBC. These reports demonstrate that antibodies to nuclear pore complex, especially antibodies to gp 210-C terminal peptides, can be surrogate markers for the progression of PBC to end-stage hepatic failure.

We recently found that PBC patients with consistently high anti-gp210-C terminal peptide antibody levels confer a high risk for the progression to end-stage hepatic failure as compared to those without those antibodies or those with initially positive but decreased to low antibody levels after treatment with UDCA (Fig. 1, 2) (5). We then conducted a retrospective multi-center cohort study of 276 biopsy-proven PBC patients. We found that male patients with positive anti-gp210 antibodies and patients with the late stage (stage III and IV) PBC have significant risk factors for death from hepatic failure/liver transplantation (LT) (Fig. 3) (49), while positive anti-centromere antibodies was a significant risk factor for the development of portal hypertension (Table 1). These studies demonstrated that PBC can be classified into two clinically different types based on the progression pattern using ANA characteristics as biomarkers.

Association of autoantibodies with HLA-DRB1

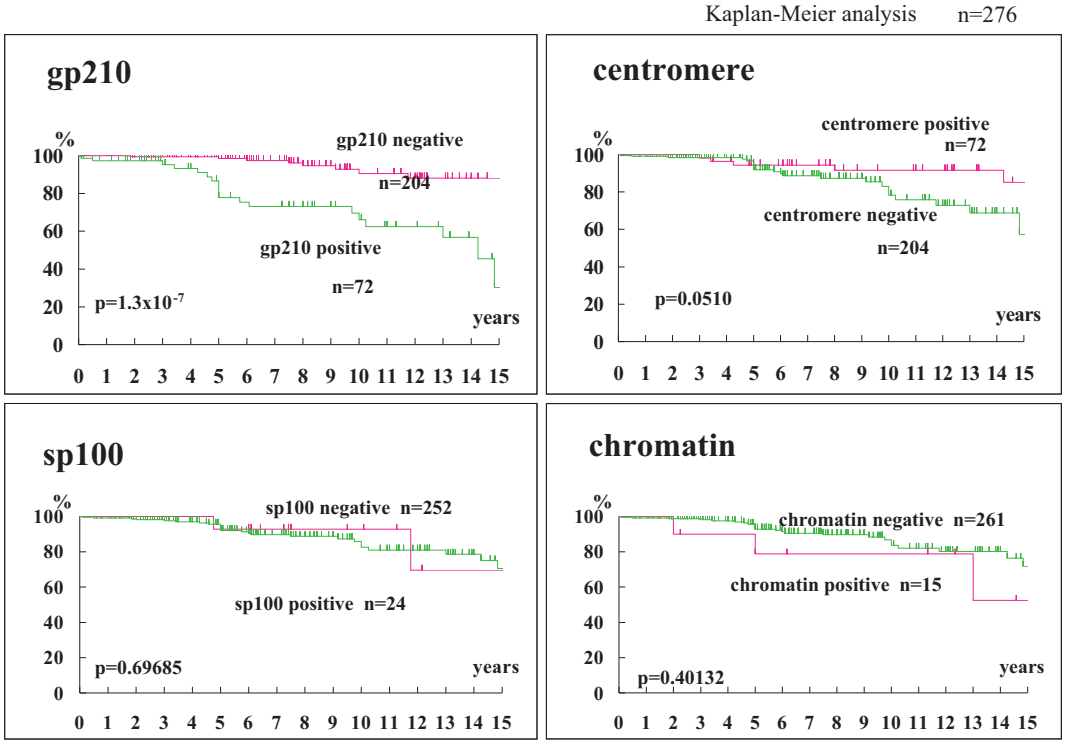
HLA-DQB1, -DPB1, -DRB1, and DRA are suggested to be associated with PBC susceptibility (30). However, only a limited number of studies have examined the association of HLA-DRB1 polymorphisms with PBC progression and autoantibody production even though it is well known that autoantibody production is influenced by specific HLA class II gene. Therefore, we studied the relationship between the progression of PBC and HLA typing in Japanese patients, and revealed that HLA-DRB1 polymorphisms are significantly associated with not only disease progression but also with antinuclear antibody production (Table 2) (50).

HLA-DRB1*0405 and *0803 were shown to predispose patients to anti-gp210 and anti-centromere antibody production, respectively. Stratifying patients by HLA-DRB1 alleles revealed that anti-gp210 antibodies were a strong risk factor for the progression to jaundice, regardless of the HLA-DRB 1 alleles, which makes the presence of anti-gp210 antibody in PBC itself a strong biomarker for the disease progression. On the other hand, the risk factor for the progression to portal hypertension in patients with anti-centromere antibodies differed among the different HLA types; increased risk factor for portal hypertension in HLA-DRB1*0405 and -DRB1* 0803 but not other HLADRB1 alleles. Thus, HLA-DRB1 polymorphisms are taken as the determinants of the risk that antinuclear antibodies contribute to the progression of PBC (50).



group A : anti-gp210 antibodies sustained at a high level
group B : anti-gp210 antibodies decreased to negative or low level
group C : anti-gp210 antibodies negative
(Nakamura M et al J.Hepatol 2005; 42:386-392)

Figure 2. **Liver transplantation-free survival.**



(Nakamura M et al, Hepatology 2007; 45:118-127)

Figure 3. **Survival curve depending on autoantibodies.**

Mechanism of progression in patients with positive anti-gp210 or anti-centromere antibodies

Based on histological observations of PBC livers, two main mechanisms appear to be involved in disease progression (51): first, bile duct destruction leading to chronic cholestasis, followed then by the development of fibrosis or cirrhosis and second, interface hepatitis, which leads to fibrosis and cirrhosis with a pattern that resembles cirrhosis following autoimmune or chronic viral hepatitis. The presence of anti-centromere antibodies was most significantly associated with more severe ductular reaction, whereas the presence of anti-gp210 antibodies was most significantly associated with more severe interface hepatitis and lobular inflamma-

Table 1. Risk Factors for the Progression of PBC

(unconditional step-wise logistic regression analysis) (n=217)

Factor	Type: Endpont:	Portal hypertension Varices, Ascites, HCC (OR (95% CI))	Hepatic failure Jaundice (OR (95% CI))
Sex, male		–	–
Age (one year^{-1})		1.08 (1.01 – 1.16)	–
anti-gp210 +ve		–	33.78 (5.93 – 636.75)
anti-centromere +ve		4.20 (1.31– 14.76)	–
anti-SP100 +ve		–	–
anti-chromatin +ve		–	–

(Nakamura M et al, Hepatology 2007; 45:118-127

Table 2. Association of HLA Type and Autoantibody Production and Progression of PBC

Variables	*Odds ratio (95% confidence interval)			
	Antibody-positive for:		Progression to:	
	Anti-gp210	Anti-centro	PHT	Jaundice
Sex, male				
Age(one year^{-1})		**1.05**	**1.06**	**0.95**
HLA-DRB1*0405	**1.61**			
HLA-DRB1*0803		**2.30**		
HLA-DRB1*0901			**1.78**	
HLA-DRB1*1502			**1.98**	
HLA-DRB1*1501				
HLA-DRB1*0402				

Nakamura et al. Hepatol Res. 2010

tion (49). Furthermore, there was a trend of more severe ductopenia (ductular reaction) in the late stage of anti-gp 210-positive patients (49). This suggests that two main mechanisms, bile duct destruction and interface hepatitis, are more severe in PBC patients positive for anti-gp210 antibodies as compared to those negative for anti-gp210 antibodies, thus leading to more frequent progression to end-stage hepatic failure (52, 53).

In another study we showed that positive anti-centromere antibodies are a risk factor for more severe ductular reaction, interface hepatitis and portal inflammation (49). There was a trend of less severe ductopenia in the late stage of anti-centromere-positive patients than anti-gp210-positive patients. The production of cytokines or growth factors from inflammatory cells, rather than retention of bile constituents, is suggested to be critical for the induction of the ductular reaction in PBC (54). Ductular reaction is also known to promote fibrosis via the production of transforming growth factor-β (TGF-β), monocyte chemoatractant protein-1 (MCP-1), and platelet-derived growth factor (PDGF) by proliferating ductal epithelia in chronic viral hepatitis (55, 56). Therefore, it is speculated that more severe ductular reaction, rather than severe bile duct damage or ductopenia, may play a critical role in the progression to portal hypertension in anti-centromere-positive PBC patients. However, the mechanism of bile duct damage tends to lead to chronic cholestasis and development of biliary cirrhosis even, to various degrees, in anti-centromere-positive PBC patients (57).

The mechanisms responsible for the production of particular ANAs in PBC are unclear. An increased expression of self-antigen is considered to be one of the mechanisms for failure of immunological tolerance. Enhanced expression of PDC-E2 was found on the luminal region of biliary epithelial cells (BECs) of small bile ducts in PBC livers (58). Also, the expression of sp100 is induced by inflammatory cytokines e.g. type 1 IFN (59). We also found that expression of gp210 antigens was increased on the nuclear envelope of epithelial cells of small bile ducts in PBC liver and that the intensity of gp210 staining by immunofluorescence was positively correlated with the intensity of inflammation

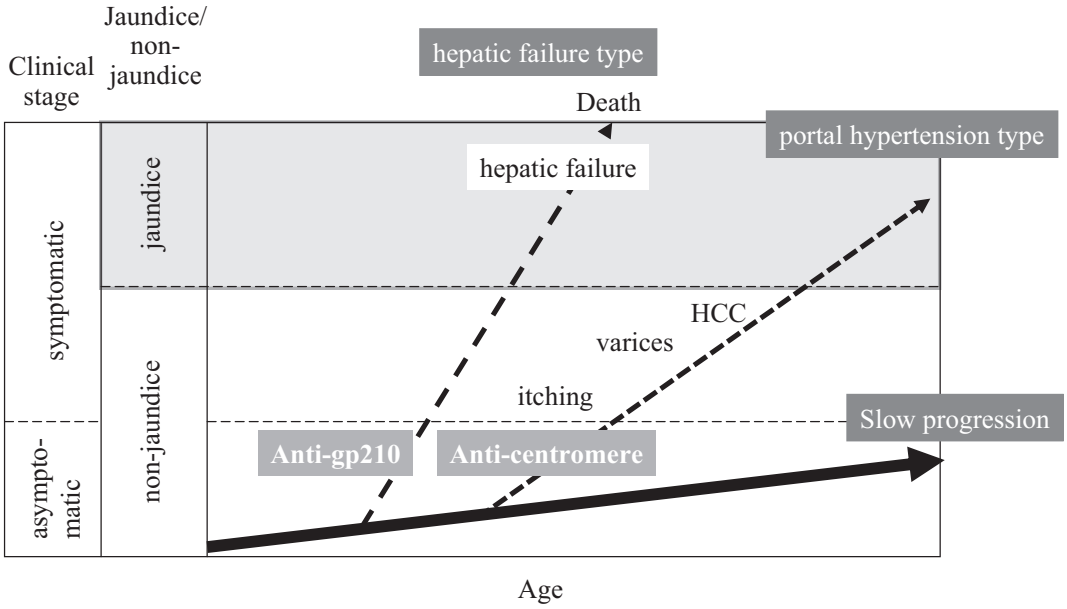


Figure 4. **Three types of progression of PBC.**

around small bile ducts (60). Taking these findings together, the consistent production of anti-gp210 could be a strong risk factor for progression to end-stage hepatic failure (60). It is indicated that gp210 is possibly a target antigen to which reactivity is important for both bile duct destruction and interface hepatitis, the two major pathological features that determine the progression of PBC (57).

Processes of apoptosis are strongly considered as mechanisms for failure of immunological tolerance in autoimmune diseases (61, 62). In fact, enhanced apoptosis was reported to be associated with bile duct loss in PBC (63). The altered apoptosis-related antigen presentation, the disruption of proteosome-dependent and/or -independent antigen processing, could play a role in the failure of immunological tolerance to nuclear autoantigens including centromere proteins (64). During apoptosis of BECs, PDC-E2 remains immunologically intact without being glutathiolated and becomes the source of the PDC-E2 apotope-"apotope" is a term coined to specify an epitope created by processes of apoptosis (65). PDC-E2 contained within the apoptotic bodies can be recognized by circulating AMA and the ensuring apotope-AMA complex then stimulates the innate immune systems in genetically susceptible individuals (65).

Molecular mimicry particularly at the T-lymphocyte level might be another mechanism for breakdown of immunological tolerance against self-antigens (66-68). According to this hypothesis, the presence of an EIExD or ExDK motif in bacterial and/or human PDC-E2, OGDC-E2, gp210, sp100 and centromere proteins could be responsible for cross-recognition by pathogenic autoreactive T cells leading on to the epitope spreading among self-antigens followed by sustained and more severe T-cell mediated inflammation (68).

These findings strongly suggest that the risk of PBC-progression is determined by the presence or absence of anti-gp210 and/or anti-centromere antibodies in an early stage of PBC. This may indicate that an autoreactive immune repertoire is prepared in an early stage of the disease process and can determine the long-term outcome of the disease. This, in turn, means we can predict a patient's prognosis using ANAs characteristics (69, 70).

Prediction of Prognosis

The natural history of PBC is divisible into four overlapping stages: a) a positive AMA with histological but no biochemical evidence of disease, the so-called 'early' PBC (9); b) abnormalities of biochemical tests, mainly elevations of serum ALP and/or γ-GTP; c) symptoms are experienced, notably itching or fatigue, and the serum bilirubin level increases beyond 2.0 mg/dL; d) the fourth final stage is marked by the serum bilirubin level exceeding 6.0 mg/dL and there are commencing features of hepatic failure, ascites and encephalopathy. The disease progression of PBC patients, however, varies greatly among individual patients. Most will remain long in stages a) and b) with a life expectancy little different from that of the general population. However, there is a group of patients in which there is rapid progression to hepatic failure with evident jaundice. Some patients develop esophageal varices without symptoms related to PBC (71). The heterogeneity of the progression has been also suggested by a histological study based on the characteristic differences according to the cirrhotic pattern of advanced PBC (72). Identifying clinical markers to predict the prognosis of PBC could be pivotal for the management of PBC patients, both for planning surgical therapy and for counseling patients.

Forms of clinical progression

Poupon described three major forms of PBC (73). The first form is patients remaining in asymptomatic stage over a

period of 10-20 years with minimal or a slow progression to biliary cirrhosis. The second form overlaps with AIH with early development of liver fibrosis and liver failure. The third form is represented by the so-called premature ductopenic variant, progressing very quickly towards cirrhosis in less than 5 years. We found that PBC disease progression can be also divided into three types; hepatic failure and portal hypertension in addition to minimal to slow progression over several years (Fig. 4) (49, 57). Hepatic failure and portal hypertension are represented by positive anti-gp210 and anti-centromere antibodies, respectively.

Natural history models

Among the several proposed predictive models tested with laboratory and clinical data, the Mayo survival model is the best validated and most often used (74). The model was originally developed to predict seven-year survival in PBC, while the model has been updated to predict two-year survival. The Mayo model is widely used to assess of the expected rate of survival without liver transplantation (75). This model is based on five independent variables including age, total serum bilirubin, albumin, prothrombin time, and severity of ascites. Of these, the serum bilirubin level is the most heavily weighted. However, the model is not suitable to predict outcome, i.e. prognosis, at an early stage and the type of progression that might occur. In addition, survival of patients who have responded to UDCA is ameliorated compared with that predicted by the Mayo model, and thus the accuracy of the model in predicting outcome in patients treated with UDCA should be re-assessed.

Prediction of prognosis by anti-gp210, anti-centromere antibodies and HLA typing

The findings in the literature suggest that patients who are male, older, and in the late stages (Scheuer's stage III, IV, if histology is available) of the disease, are at significant risk for a poor outcome at the time of diagnosis. A histological finding of interface hepatitis also predicts a poor outcome. Further, we have shown that a persistent positive test for anti-gp210 confers a strong risk for a progressive clinical course and thus is a good index of outcome (5, 49). We recommend that the titer of the antibody should be re-evaluated 3 to 6 months after the initiation of UDCA therapy when a PBC patient shows positivity for anti-gp210 antibody. If the anti-gp210 antibody titer decreases and becomes negative, the patient can expect a good prognosis whereas the patient whose anti-gp210 antibody remains high cannot expect a good prognosis, progressing with high probability to hepatic failure.

HLA typing combined with autoantibody characteristics can also provide the prediction of a patient's outcome; patients with HLA-DRB1*0405 and -DRB1*0803 are predisposed to develop anti-gp210 and anti-centromere antibodies, respectively. When a patient with HLA-DRB1*0405 or -DRB1*0803 is positive for anti-centromere antibodies, that patient is predisposed to the progression to portal hypertension but this does not pertain to other HLADRB1 alleles. Both HLA-DRB1*1502 and *0901 predispose to non-jaundice-type progression, as does anti-gp210, although the latter is a strong indicator for progression to jaundice, irrespective of HLA-DRB1 alleles (50). Thus, it appears that HLA may contribute to but not entirely explain ANA specificity and disease expression in PBC. Accordingly future genome-wide association studies should stratify cases according to autoantibody specificities to obtain further genetic data pertinent to this question.

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

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