Table 2 The baseline characteristics of the patients with primary biliary cirrhosis and autoimmune hepatitis (PBC-AIH) overlap, PBC and AIH

	PBC-AIH overlap ($n = 33$)	PBC ($n = 89$)	AIH ($n = 44$)
Sex, M/F	2/31	10/79	5/39
Age (year), mean ± SD (min-max)	54.6 ± 11.9 (34–84)*	59.9 ± 11.9 (29–91)	58.4 ± 15.0 (16–79)
Mean follow-up (years), mean ± SD (min-max)	6.1 ± 5.2 (0.3–16.3)	5.3 ± 4.6 (0.0–18.1)	8.0 ± 6.5 (0.0–22.8)
Presence of other autoimmune diseases			
The patient (yes/no)	9/24	18/71	10/34
Family members (yes/no/unknown)	1/24/8	N/A	N/A
BMI, mean ± SD (min-max)	23.0 ± 4.0 (17.6–34.4)	N/A	N/A
Presence of any symptom (yes/no)	10/23*	13/76	9/35
AST (IU/L), median (min-max)	155 (33–1171)**	42 (19–298)	202 (18–856)
ALT (IU/L), median (min-max)	230 (23–1490)**	45 (14–307)	287 (21–973)
ALP (IU/L), median (min-max)	579 (171–6866)†	490 (170–1659)	352 (198–962)
GGT (IU/L), median (min-max)	203 (30–1161)††	182 (21–2160)	67 (22–265)
Albumin (g/dL), median (min-max)	3.9 (1.8–4.8)	4.0 (2.6–4.7)	3.9 (2.2–4.6)
Total bilirubin (mg/dL), median (min-max)	1.15 (0.3–20.5)	0.70 (0.2–3.2)	1.30 (0.2–24)
IgG (mg/dL), median (min-max)	2516 (1260–5150)**	1550 (949–2490)	2786 (1065–6420)
IgM (mg/dL), median (min-max)	395 (71–2340)**††	271 (84–1058)	221 (65–576)
AMA (positive/negative)	29/4††	80/9	4/40
ANA or SMA (positive/negative)	29/4**	51/38	43/1

N/A, not applicable.
*Values significantly different from patients with PBC; *$P < 0.05$, **$P < 0.01$.
†Values significantly different from patients with AIH; †$P < 0.05$, ††$P < 0.01$.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, anti-mitochondrial antibodies; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyltranspeptidase; SD, standard deviation; SMA, smooth muscle antibody.

0.3 ± 0.7 and 1.5 ± 0.9, respectively. The grades of three PBC-like features, bile duct injury, bile duct loss and atypical ductular reaction, were 1.4 ± 1.0, 0.9 ± 0.9, and 0.9 ± 0.9, respectively (Table 4). Thus, interface hepatitis was the most remarkable histological finding in PBC-AIH overlap cases, followed by plasma cell infiltration and bile duct injury. The superiority of AIH-like features such as interface hepatitis compared to PBC-like features was also confirmed by the analyses of necroinflammatory activities (Fig. 1a,b). The mean of CA and HA were 1.27 ± 0.98 and 2.27 ± 0.67, respectively, and thus the HA score was significantly higher than the CA score ($P < 0.001$).

Table 3 Interpretation of the revised International Autoimmune Hepatitis Group (IAIHG) aggregate scores at post-treatment

	AMA positive: −4	AMA negative: ±0
Not AIH	6	0
Probable AIH	19	11
Definite AIH	8	22

Treatment and prognosis

Initial treatment regimens in 33 patients with PBC-AIH overlap is shown in Table 5. As an initial treatment regimen, UDCA monotherapy or UDCA+bezafibrate were administered in 16 patients, and corticosteroids were initially chosen without UDCA in six patients and with UDCA in 10 patients. The initial treatment included UDCA in 26 patients and corticosteroids in 16, respectively. During the follow-up period, corticosteroids had been added to UDCA in seven patients in whom the initial treatment was UDCA monotherapy, and, by contrast, UDCA had been added to corticosteroids in four patients in whom corticosteroids monotherapy was selected as an initial treatment regimen. Thus at the end of the follow-up period, corticosteroids with and without other drugs were used in 23 patients out of 32 patients (72%) with clear history of treatment regimens, while nine patients did not require corticosteroids treatment (Table 5). These therapeutic regimens

Table 4 Histopathological findings of primary biliary cirrhosis and autoimmune hepatitis (PBC-AIH) overlap

AIH features findings	Grade	*n*	PBC features findings	Grade	*n*
Interface hepatitis			Bile duct injury		
	0	1		0	6
	1	7		1	14
	2	15		2	8
	3	10		3	5
Mean ± SD	2.0 ± 0.8		Mean ± SD	1.4 ± 1.0	
Rosette formation			Bile duct loss		
	0	25		0	14
	1	6		1	11
	2	1		2	6
	3	1		3	2
Mean ± SD	0.3 ± 0.7		Mean ± SD	0.9 ± 0.9	
Plasma cell infiltration			Atypical ductular reaction		
	0	3		0	14
	1	16		1	11
	2	8		2	6
	3	6		3	2
Mean ± SD	1.5 ± 0.9		Mean ± SD	0.9 ± 0.9	

achieved no deterioration of liver function without any symptom in 30 patients, including nine patients only with UDCA monotherapy or UDCA+bezafibrate (Table 5). In two patients, in both of which combination therapy of UDCA and corticosteroids were administered, deterioration of liver function as well as development of symptoms were observed; jaundice in one and hepatic encephalopathy in one. Nevertheless, although one female patient died due to ovarian cancer, neither liver-related death, nor liver transplantation had been noted during the follow-up period.

Application of Paris criteria and simplified IAIHG scoring system for diagnosis of AIH features to the training set of PBC-AIH overlap

We randomly divided the 33 PBC-AIH overlap cases into two groups; training set ($n = 17$) and validation set ($n = 16$). There was no significant difference in each value between two sets (Table 6). Then, we applied two criteria, Paris criteria[17] and the simplified IAIHG scoring system,[15] which have been frequently used for diagnosis of PBC-AIH overlap, to the training set. In Paris criteria, two out of three items: (i) serum ALT levels >5xULN;, (ii) serum IgG levels >2xULN or anti-smooth muscle antibody (SMA) positive; and (iii) moderate or severe periportal or periseptal lymphocytic piecemeal necrosis, are needed to fulfill AIH features.[17] In 17 patients in the training set, the number of patients who met 0, 1, 2, and 3 items was 0, 1, 9, and 7, respectively, and thus 16 out of 17 (94%) met at least two items and were definedj as having AIH features using Paris criteria (Table 7). However, among four patients who did not require corticosteroids therapy, three were diagnosed as having AIH features using Paris criteria (Table 7). The sensitivity and specificity of Paris criteria for prediction of necessity of corticosteroids treatment was 92% and 25%, respectively.

On the other hand, the simplified IAIHG scoring system included four items; detectable autoantibodies, serum IgG level, liver histology and absence of viral hepatitis. In the current study we adopted the HA scores as liver histology. Since serum IgG at presentation was missing in one patient in the training set, we applied the simplified scoring system to the 16 patients in the training set. The simplified scores in 16 patients ranged from 4 to 8, and 13 out of 16 patients (81%) were diagnosed as having AIH features using the simplified scoring system (Table 7). Meanwhile, among four patients who did not need corticosteroids administration, only one patient was diagnosed as AIH (Table 7), and thus the sensitivity and specificity of simplified scoring system for prediction of requirement of corticosteroids was 92% and 75%, respectively, the latter being higher than that of the Paris criteria.

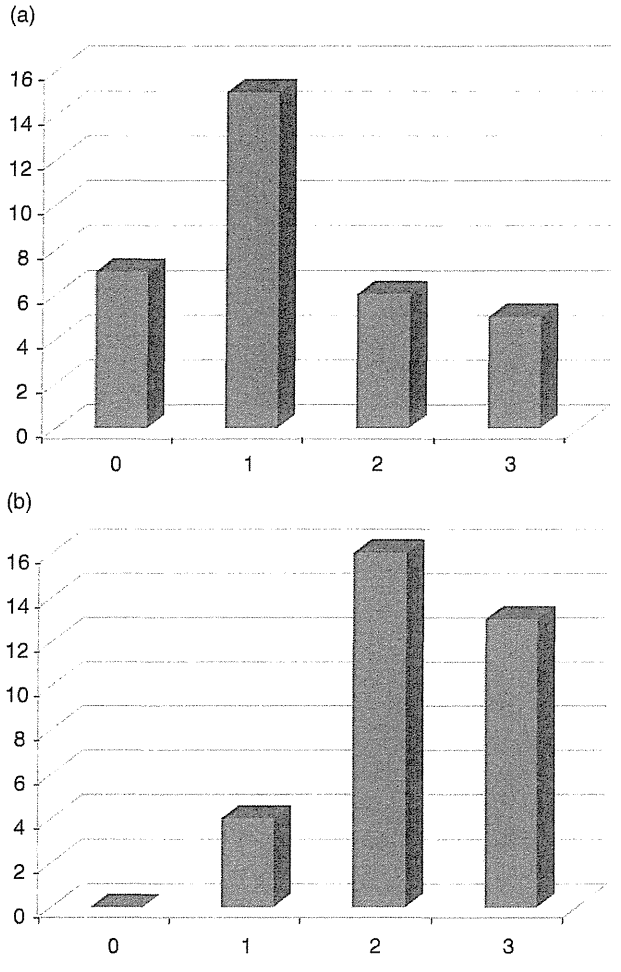

Figure 1 The distribution of necroinflammatory activities, the CA (cholangitis activity) score (a) and the HA (hepatitis activity) score (b) in 33 primary biliary cirrhosis and autoimmune hepatitis (PBC-AIH) overlap cases. The mean of the CA score and the HA score were 1.27 ± 0.98 and 2.27 ± 0.67, respectively, and the HA score was significantly higher than the CA score ($P < 0.001$).

Application of simplified IAIHG scoring system for diagnosis of AIH features to the validation set of PBC-AIH overlap and PBC/AIH alone

Therefore we applied the simplified scoring system to the validation set of PBC-AIH overlap, as well as patients with PBC or AIH alone, and the results are shown in Table 8. Among 16 patients in the validation set, 11 and five patients were diagnosed as AIH and not AIH using the simplified scoring system, respectively, and the sensitivity and specificity for corticosteroid use was 91% and 80%, both of which were supposed to be equally satisfactory and acceptable. In 44 patients with AIH alone, 38 patients (86%) were diagnosed as having AIH. The number of patients with corticosteroid use in the follow-up period was 35, and the sensitivity and specificity for corticosteroid use was 94% and 44%. By contrast, only three patients in 89 patients with PBC (3%) were considered to have the AIH feature using these criteria.

DISCUSSION

IN THIS RETROSPECTIVE study, we enrolled patients with PBC-AIH overlap as much as possible with a nation-wide scale in Japan and first aimed to describe clinical features of them. Among 1081 PBC and 597 AIH patients from eight referral centers in Japan, we identified 33 PBC-AIH overlap cases followed up 6.1 years on average with clinical, biochemical, immunological and histological dataset at diagnosis as well as information about treatment and prognosis. The number of 33 is quite large compared to other similar studies published in this decade,[12,16,18,19,21–23,26–28] and this is an advantage of the current study. It seems that clinical, biochemical and immunological features of PBC-AIH overlap collected in this study are comparable to those in previous studies.[12,16,18–23,26,28] For instance, mean age at presentation was 50–55 years except for two studies (41 and 44 years),[18,22] marked female predisposition, elevation of serum ALT/AST levels, presence of various autoantibodies (AMA/ANA/SMA), and absence of viral markers. In addition we included patients with PBC or AIH alone as controls in this study, 89 PBC and 44 AIH patients.

Table 5 Treatment regimens for thirty three primary biliary cirrhosis and autoimmune hepatitis (PBC-AIH) overlap at presentation and at the end of follow-up

	Initial treatment	At the end of follow-up
UDCA monotherapy	15	8
UDCA + bezafibrate	1	1
Corticosteroids monotherapy	6	2
UDCA + corticosteroids	8	16
UDCA + corticosteroids + azathioprine	1	1
UDCA + corticosteroids + bezafibrate	1	4
Unknown	1	1
Including UDCA	26	30
Including corticosteroids	16	23

UDCA, ursodeoxycholic acid.

Table 6 The baseline characteristics of the patients with primary biliary cirrhosis and autoimmune hepatitis (PBC-AIH) overlap; training set and validation set†

	Training set ($n = 17$)	Validation set ($n = 16$)
Sex, M/F	0/17	2/14
Age (year), mean ± SD [min-max]	55.1 ± 12.5 [34–77]	54.1 ± 11.5 [38–84]
Mean follow-up (years), mean ± SD [min-max]	6.1 ± 5.0 [0.3–14.6]	6.0 ± 5.5 [0.3–16.3]
Presence of other autoimmune diseases		
The patient (yes/no)	5/12	4/12
Family members (yes/no/unknown)	0/12/5	1/12/3
BMI, mean ± SD [min-max]	22.8 ± 4.5 [18.3–34.4]	23.2 ± 3.6 [17.6–32.2]
Presence of any symptom (yes/no)	6/11	4/12
AST (IU/L), median[min-max]	314 [44–1171]	146 [33–753]
ALT (IU/L), median[min-max]	343 [42–1490]	202 [23–1127]
ALP (IU/L), median[min-max]	539 [197–6866]	607 [171–2220]
GGT (IU/L), median[min-max]	204 [33–1161]	152 [30–759]
Albumin (g/dL), median[min-max]	4.0 [2.6–4.5]	3.9 [1.8–4.8]
Total bilirubin (mg/dL), median[min-max]	1.20 [0.3–20.5]	1.10 [0.5–8.5]
IgG (mg/dL), median[min-max]	2217 [1645–3950]	2765 [1260–5150]
IgM (mg/dL), median[min-max]	381 [71–2340]	397 [129–2058]
AMA (positive/negative)	14/3	15/1
ANA (positive/negative)	15/2	14/2

†There was no significant difference in each value between training and validation sets.

The comparison among PBC, AIH, and PBC-AIH overlap suggested similar characteristics of PBC-AIH overlap as previously reported; higher AST/ALT/IgG compared to PBC, and higher ALP/GGT/IgM compared to AIH.[19,21,23]

Although liver histology at diagnosis of PBC-AIH overlap was not intensively analyzed in previous studies, we in the current study were able to examine liver histology of all PBC-AIH overlap at presentation in detail. We demonstrated that AIH-like histological findings such as interface hepatitis were more dominant compared to PBC-like features. Indeed previous reports have shown that prevalence of interface hepatitis was reported to be high, 69%,[12] 92%,[22] and 93%,[16] which were coincident with our results (97%). In addition we have shown that the scores of AIH-like histological features as well as the HA score were significantly elevated compared with those of PBC-like features (Table 4) and the CA score (Fig. 1a,b). The superiority of AIH-like features of liver histology in PBC-AIH overlap cases has not been clearly demonstrated previously. Presumably this is because of presence of AMA for diagnosis of PBC, and by contrast, lack of specific markers for diagnosis of AIH.

Table 7 Application of Paris criteria and the simplified scoring system for diagnosis of autoimmune hepatitis (AIH) features and corticosteroids use to the training set

		Paris		Simplified	
		Yes	No	Yes	No
Overlap; training set	17†	16	1	13	3
Corticosteroids (+)	12‡	11	1	11	1
Corticosteroids (−)	4	3	1	1	3

†1 In one patient categorized in "corticosteroids (+)", serum IgG level was missing and simplified score was not calculated.
‡2 The information about treatment was missing in one patient.

Table 8 Application of the simplified scoring system for diagnosis of autoimmune hepatitis (AIH) features and corticosteroids use to the validation set, primary biliary cirrhosis (PBC) and AIH

		Simplified	
		Yes	No
Overlap; validation set	16	11	5
Corticosteroids (+)	11	10	1
Corticosteroids (−)	5	1	4
AIH	44	38	6
Corticosteroids (+)	35	33	2
Corticosteroids (−)	9	5	4
PBC†	89	3	86

†No patients with PBC were administered corticosteroids.

We could assume that clinicians tend to depend on the presence of AMA for diagnosis of PBC features, and depend on histological findings more intensely for diagnosis of AIH features.

We are of course aware that, however, inclusion/diagnostic criteria for PBC-AIH overlap are the most challenging problems to be discussed in the current study as well as in other studies describing PBC-AIH overlap. We tentatively adopted the "modified" revised IAIHG scoring systems, i.e. with dismissal of negative allocation of AMA positivity as −4, and 1 and 3 cases in the training set were not diagnosed as PBC-AIH overlap using Paris criteria and the simplified scoring system, respectively (Table 7). It should be kept in mind that PBC-AIH overlap has not been precisely defined and numbers of controversies on the etiopathogenesis still remain to be solved. A recent position statement from the IAIHG clearly indicated that the definition of diagnostic criteria for overlap conditions could only be arbitrary, and patients with overlapping features were not considered as being distinct diagnostic entities.[29] We partly agree with this statement, and do not aim in this study to seek which diagnostic criteria are effective for establishing the diagnosis of PBC-AIH overlap. Nevertheless, we believe that treatment guidelines or rationales for corticosteroids use in PBC-AIH overlap are absolutely required in the clinical setting, although the position statement remarked that combination therapy of UDCA and immunosuppressive drugs was not evidence-based.[29] It has been frequently demonstrated by several clinical studies that some patients with PBC-AIH overlap may benefit from combination therapy,[12,18,21,26,30] and in spite of the apparent efficacy of corticosteroids clinicians are usually reluctant to administer corticosteroids to their patients due to several well-known adverse effects which could be very serious in the long run. Therefore, our aim in this study is to address a rationale for corticosteroids administration in patients with "indefinitely" defined PBC-AIH overlap.

Although the follow-up period of the enrolled patients was 6.1 years and not sufficiently long, the prognosis of these 33 patients were excellent without any liver-related death or transplantation. Thus, we assumed as a working hypothesis that all treatment strategies for these patients were ideal and correct, and retrospectively sought which diagnostic criteria, Paris criteria and the simplified criteria, would well identify those who later required corticosteroids therapy using clinical parameters at presentation. In this regard, Paris criteria identified 16 out of 17 patients in the training set as having AIH-features but three out of 16 did not required corticosteroids therapy, and therefore it seems that "overdiagnosis" was raised using Paris criteria. The sensitivity and specificity for corticosteroids use were 92% and 25% with Paris criteria, the latter being very low and not acceptable. On the other hand, "overdiagnosis" was relatively suppressed using the simplified scoring system, and the sensitivity and specificity were 92% and 75%; the sensitivity was comparable and the specificity was elevated compared to Paris criteria. In the validation set, the sensitivity and specificity of the simplified scoring system for corticosteroid use was 91% and 80%, both of which were equally satisfactory and acceptable. We assume that the improved specificity using the simplified criteria is partly explained by the fact that the simplified scoring system was originally designed for early diagnosis of AIH, which allows clinicians to start treatment with immunosuppressive drugs earlier.[15] In other words the simplified scoring system was beneficial for selection of patients requiring immunosuppressive drugs, and therefore may be also effective to identify patients who need corticosteroids administration among the cohort enrolled in the current study. Additionally, it is of note that the "overdiagnosis" of patients with PBC alone as indicated for corticosteroid use is preventable. Only three patients in 89 patients with PBC (3%) were considered to have AIH feature using this criteria. Although the specificity for corticosteroid use in patients with AIH alone appears to be low (44%), this is because corticosteroids had not been administered to some patients with AIH in our cohort, in whom indications of corticosteroids were surely present, due to various clinical reasons.

In conclusion, we performed a nation-wide retrospective study for description of PBC-AIH overlap, and addressed a rationale for corticosteroids administration basically consisting of the simplified scoring system for AIH. Clearly a large scale prospective study is warranted to investigate the usefulness of this strategy in the future.

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CLINICAL STUDIES

Hepatocellular carcinoma and survival in patients with autoimmune hepatitis (Japanese National Hospital Organization-autoimmune hepatitis prospective study)

Kiyoshi Migita[1], Yukio Watanabe[1], Yuka Jiuchi[1], Yoko Nakamura[1], Akira Saito[1], Michiyasu Yagura[1], Hajime Ohta[1], Masaaki Shimada[1], Eiji Mita[1], Taizo Hijioka[1], Haruhiro Yamashita[1], Eiichi Takezaki[1], Toyokichi Muro[1], Hironori Sakai[1], Makoto Nakamuta[1], Seigo Abiru[1], Atsumasa Komori[1], Masahiro Ito[1], Hiroshi Yatsuhashi[1], Minoru Nakamura[1] and Hiromi Ishibashi[1] Japanese NHO-Liver-network study group

Clinical Research Center, NHO Nagasaki Medical Center, Omura, Japan

Keywords
autoimmune hepatitis – cirrhosis – hepatocellular carcinoma – multicentre cohort study – outcome

Correspondence
Kiyoshi Migita, MD, Clinical Research Center, NHO Nagasaki Medical Center, Kubara 2-1001-1, Omura 856-8652, Japan
Tel: +81 957 52 3121
Fax:+81 957 53 6675
e-mail: migita@nmc.hosp.go.jp

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Abstract

Background/Aims: Although the outcome of autoimmune hepatitis (AIH) is generally good, the natural course and likelihood of progression to cirrhosis or hepatocellular carcinoma (HCC) remain undefined, and may vary by region and population structure. Our aims were to evaluate risk factors that contribute to poor outcome and particularly development of HCC in a prospective multicentric cohort study of AIH. *Methods:* The study group comprised 193 Japanese patients with AIH who were prospectively followed up at annual intervals between 1995 and 2008. The mean follow-up period was 8.0 ± 4.5 years. *Results:* Twenty-one (10.9%) patients had cirrhosis at presentation and a further 15 (7.8%) developed cirrhosis during the follow-up period. Survival rates were 94.2% at 10 years and 89.3% at 15 years. HCC was diagnosed in seven of the 193 patients. The presence of cirrhosis at presentation was a risk factor for HCC according to a Cox proportional hazard model, and the HCC-free survival rate was significantly lower in those with cirrhosis compared to those without cirrhosis according to Kaplan–Meier analysis. *Conclusions:* Although the outcome of AIH is as good if not better among Japanese than for other populations, there was an increased risk of HCC in these patients. Cirrhosis at presentation was predictive of development of HCC in AIH in Japan.

Autoimmune hepatitis (AIH) is a chronic inflammatory disorder, dependent in part on autoimmune reactivity, that can cause cirrhosis and end-stage liver disease (1, 2). Current descriptions of features of AIH, derived mostly from Caucasian patients, cite a generally good outcome with 10- and 20-year survivals more than 80% (3). Similarly, the outcome among Japanese patients with AIH is generally good with a 10-year survival rate reported as 90% (4). In general, the natural history and course of AIH are largely defined by the degree of inflammatory activity at the onset of disease and the presence or development of cirrhosis (5). Hepatocellular carcinoma (HCC) complicating AIH is reported (6) but occurs rarely among Caucasian populations secondary to AIH (7): the true incidence remains uncertain and factors contributing to development of HCC in AIH are not fully elucidated. To better understand the natural history and outcome of AIH, and to establish comparisons of AIH among Japanese and Caucasian patients, a nation-wide multicentre cohort study was developed, and herein, we describe the clinical presentation, course and efficacy of treatment of 193 consecutive AIH patients enrolled in the Japanese National Hospital Organization (NHO)-AIH register. We particularly assessed risk factors for a fatal outcome and development of HCC.

Patients and methods

Study population

There were 212 patients initially enrolled in the register of the Japanese National Hospital Organization (NHO) liver-network study, contributed to medical facilities in Japan. Of these 212 patients, 193 were retained and prospectively followed between 1995 and 2008 as a multicentre cohort population. All patients satisfied the 1999 revised criteria of International Autoimmune Hepatitis Group (IAIHG) for a diagnosis of definite (114 cases) or probable (79 cases) AIH (8). Patients were excluded

from study if there was histological evidence of cholangitis or non-alcoholic steatohepatitis. Also, patients who were positive for hepatitis B virus (HBV)-surface antigen (HBsAg) or hepatitis C virus (HCV)-RNA were excluded and other causes of liver disease, such as excess alcohol, or drugs had been excluded by appropriate history and investigations. The study protocol was approved by the Ethics Committees of all institutes.

Clinical and histological assessments

Follow-up assessments were made at annual intervals. Standard laboratory tests of liver inflammation and function were measured at each assessment. Liver tissue from percutaneous biopsy performed at the referring facility was available for the majority of the patients at the time of entry (143/193, 74.1%) and at subsequent follow-up examination for some (39/193, 20.2%). The histological variables examined included degree of fibrosis (0; absent, 1; expansion of fibrosis to parenchyma, 2; portal-central or portal-portal bridging fibrosis, 3; presence of numerous fibrous septa, 4; multi-nodular cirrhosis). The histological diagnosis of cirrhosis required loss of normal lobular architecture, reconstruction of hepatic nodules and presence of regenerative nodules (9). Biopsy samples from AIH patients developing to HCC were examined in a blinded fashion by a dedicated pathologist (MI). Anti-nuclear antibodies (ANA) and smooth muscle antibodies (SMA) were measured by indirect immunofluorescence on HEp-2 cell respectively and cut-off titres for positivity were 1:40. Clinical relapse was defined as an increase of serum ALT levels to beyond three-fold of the upper limit of normal range (ULN) (10). Asymptomatic patients or patients with lower serum aminotransferase, total bilirubin or IgG were managed with ursodeoxycholic acid (UDCA) therapy alone, which was demonstrated to be effective in Japanese patients with type I autoimmune hepatitis (11).

Variables at study entry

Demographic and other characteristics of the 193 retained patients were recorded as a data-base at the initial assessment. Data included gender, age at diagnosis, time of onset of symptoms or other evidence of liver disease, markers of infection with hepatitis viruses HBV and HCV, alcohol intake, coexisting autoimmune diseases, serum levels of ALT, AST, alkaline phosphatase and bilirubin, platelet count and prothrombin time.

Occurrence of hepatocellular carcinoma

Abdominal ultrasound and serum alpha-fetoprotein determinations were performed annually. Viral hepatitis was excluded by testing for HBsAg and HCV-RNA by polymerase chain reaction (PCR). Subjects with antibodies to HCV were subsequently screened for HCV-RNA using nested PCR. A diagnosis of HCC was made based on the typical patterns on imaging studies, such as early-phase hyperattenuation and late-phase hypoattenuation by dynamic computerized tomography, magnetic resonance imaging and finally, by ultrasonography-guided tumour biopsy.

Statistical analysis

For quantitative data, analysis was performed using a Mann–Whitney test for comparison of two independent groups. Differences in proportions were analysed by the Fisher's exact test when the number of subjects was <5, and the chi-squared test for 2×2 tables when the number of subjects was >5. Prognostic factors for HCC were analysed using the univariate and multivariate Cox proportional hazard model with SPSS software (Chicago, Illinois, USA). The *P*-values of entering variables for multivariate Cox proportional hazard model were <0.1. Survival, related to follow-up time, was analysed using the Kaplan–Meier method and compared using the log-rank test. A value of $P \leq 0.05$ was considered statistically significant.

Results

Baseline data at entry

Of the original 212 recruited patients registered as AIH, 19 were excluded from analysis owing to loss of follow-up (Fig. 1). The retained 193 were considered eligible for the study. Table 1 presents the demographic data for the cohort at entry. The age at diagnosis ranged from 16 to 84 years (mean, 56.6 ± 13.9 years), greater than that in earlier studies on Caucasian patients, and female patients predominated (91.7%). In 51 patients (26.4%) there was concurrent symptomatic autoimmune disease, notably Hashimoto thyroiditis in 11, rheumatoid arthritis in 20, systemic lupus erythematosus in 6, Sjögren's syndrome in 14 and systemic sclerosis in 2. Regarding tests for autoantibodies, data for SMA were lacking in 105 and for ANA in 5. Of those tested, 158 (84.0%) gave positive tests (titre >1:40) for ANA and 36 (40.9%) for SMA. Regarding treatment, 144 patients (74.6%) had been treated with prednisolone, and 43 (22.3%) with ursodeoxycholic acid alone. Relapse occurred in 47 (24.4%) during the follow-up period.

Patient outcome and survival

Seven patients (3.6%) died as a result of complications of AIH. Liver-related death (HCC 2, ruptured oesophageal varices 1, hepatic failure 1) was confirmed in four AIH patients with cirrhosis at presentation (19.0%), one patient (hepatic failure) who subsequently developed cirrhosis during follow-up (6.7%) and one patient (fulminant hepatitis) without cirrhosis during the follow-up period (Fig. 1, Table 2). The overall survival in the AIH patients is shown in Figure 2. The calculated

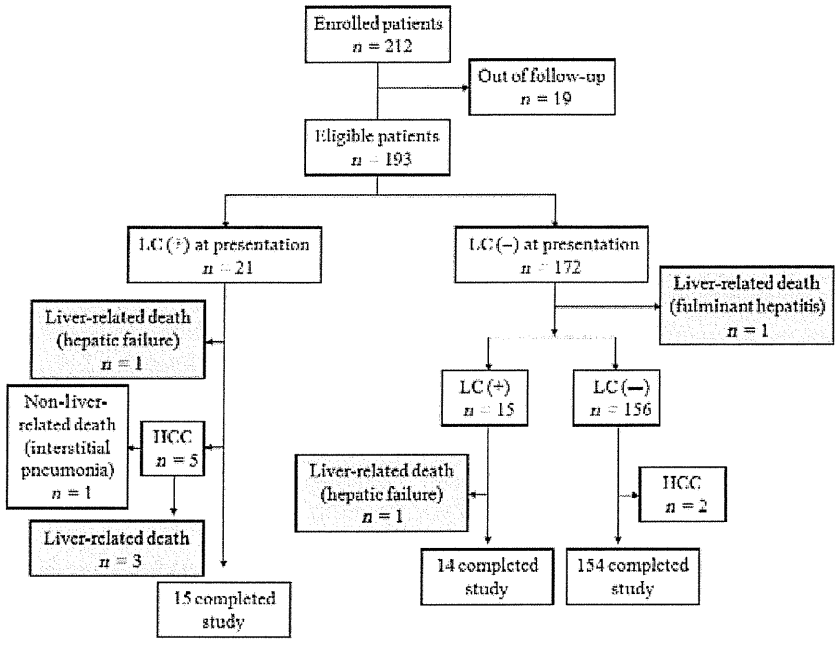

Fig. 1. Flow diagram of patient selection and clinical outcome of autoimmune hepatitis (AIH) patients in the present cohort study. C; cirrhosis, HCC; hepatocellular carcinoma.

Table 1. Baseline characteristics of AIH patients

	n = 193
Gender (male/female)	16/177
Mean age at presentation (years)	56.6 ± 13.9 (16–84)
Mean age	
Age ≥ 60year	98 (50.8%)
Age<60year	95 (49.2%)
Other autoimmune diseases	51 (26.4%)
Mean follow-up (years)	8.0 ± 4.5 (0.1–21)
Baseline Laboratory Values	
AST (<40IU/L)	392.00 ± 450.65 (29–2718)
ALT (<40IU/L)	408.55 ± 421.21 (18–2020)
ALP (<112U/L)	453.18 ± 270.04 (112–2135)
Bilirubin (mg/dl)	3.95 ± 5.66 (0.27–31.8)
Albumin (3.5–5.0g/L)	3.76 ± 0.61 (2.00–5.10)
IgG (500–1300mg/dl)	2517.49 ± 913.43 (210.2–5221)
Platelets (15–40×10^4μl)	19.25 ± 8.02 × 10^4 (2.00–57.00 × 10^4)
ANA+ (≥ 1:40)	158/188 (84.0%)
SMA+ (≥ 1:40)	36/88 (40.9%)
Cirrhosis at presentation	21 (10.9%)
Received treatment	
Mean PSL (mg/day)	28.71 ± 72.98 (0–1000)
PSL ≥ 20mg	126 (65.3%)
PSL alone	100 (51.8%)
PSL+UDCA	42 (21.8%)
PSL+Aza	2 (1.0%)
UDCA alone	43 (22.3%)
Relapse	47 (24.4%)

ALP, alkaline phosphate; ALT, alanine aminotransferase; ANA, antinuclear antibody; AST, aspartate aminotransferase; Aza, azathioprine; PSL, prednisolone; SMA, anti-smooth muscle antibody; UDCA, ursodeoxy cholic acid.

Table 2. Fatal outcome of patients with type 1 autoimmune hepatitis

	Cirrhosis at entry (*n* = 21)	Developed cirrhosis (*n* = 15)	No cirrhosis (*n* = 157)
Deaths (*n*)	5	2	5
Liver-related	4	1	1
HCC	2	0	0
Ruptured oesophageal varices	1	0	0
Fulminant hepatitis	0	0	1
Liver failure	1	1	0
Non-Liver-related	1	1	4
CVA	0	0	3
Lymphoma	0	1	0
Lung cancer	0	0	1
Interstitial pneumonia	1	0	0
Follow-up after cirrhosis (year)	10.0 ± 4.8	4.3 ± 3.9	NA
Total follow-up (year)	10.0 ± 4.8	9.5 ± 4.6	7.6 ± 4.4

Note: Numbers in parentheses represent percentages. Data are expressed as mean ± SD.
HCC, hepatocellular carcinoma; CVA, c erebrovascular accident.

survival for the entire cohort was 94.2% at 10 years and 89.3% at 15 years.

Development of HCC

Hepatocellular carcinoma was diagnosed in as many as seven of the 193 (3.6%) patients with AIH, in five

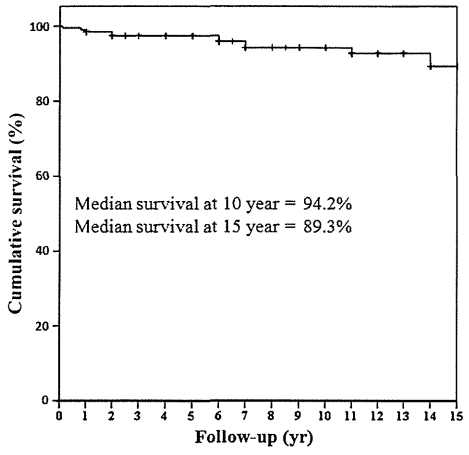

Fig. 2. Survival curve of Japanese patients with AIH. The 10-year survival was 94.2% and 15-year survival was 89.3%.

female patients (2.6%) and in two male patients (1.0%). The mean age at diagnosis was 62.0 ± 10.5 years (range, 43–73 years; median 65 years). Of the seven patients who developed HCC, five had antecedent cirrhosis (mean duration of cirrhosis, 9.0 ± 3.5 years; range, 4–12 years; median 11 years); two who developed HCC did not have antecedent cirrhosis (Fig. 1). Two patients with cirrhosis and HCC died within 2 years after the diagnosis of HCC and three were surviving at the last follow-up. HCC developed in seven patients. Table 3 summarizes the clinical features, and outcome of these HCC patients. At diagnosis of HCC, five patients had cirrhosis and the remaining two patients did not progress to cirrhosis. Overall, four patients died, among which two patients died of HCC, one of ruptured oesophageal varies and one of interstitial pneumonia. The mean survival time from HCC diagnosis was 3.2 ± 2.2 years in these four patients. The remaining three patients still alive and the mean duration from HCC diagnosis to the final surveillance was 5.0 ± 1.3 years.

Initial clinical features and laboratory data at diagnosis of AIH were compared between patients with and without HCC (Table 4). In patients who developed HCC, the frequencies of the association with cirrhosis were significantly higher. Also lower platelet counts, total bilirubin and ALT were observed in the group with HCC. Using time-dependent univariate analysis (Cox proportional hazard model), the following variables at

Table 3. Clinical features of 7 patients who developed HCC

Patient no	Case1	Case2	Case3	Case4	Case5	Case6	Case7
Age at diagnosis (years)	72	60	73	43	55	65	66
Gender	Female	Female	Female	Female	Male	Male	Female
Labo data at diagnosis							
Platelet (/μl)	126000	196000	172000	94000	129000	135000	44000
AST/ALT (IU/ml)	244/86	102/55	140/107	57/44	95/185	100/159	80/66
Cirrhosis at presentation	(+)	(–)	(+)	(+)	(+)	(–)	(+)
Liver histology at presentation							
Grading A score	NT	NT	A2	A2	A2	A2	A2
Staging F score	NT	NT	F3	F4	F4	Fl	F3
Other autoimmune diseases	SLE	RA	(+)	Chronic thyroiditis	(–)	(–)	(–)
Initial treatments	PSL30 mg/day	PSL2.5 mg/day	PSL30 mg/day	PSL40 mg/day	UDCA600 mg/day	PSL30 mg/day UDCA60 0 mg/day	PSL30 mg/day UDCA300 mg/day
Age at HCC diagnosis (years)	84	72	79	54	67	71	70
Duration from AIH diagnosis (years)	11.5	11.1	5.7	11.5	11.5	5.9	4.0
Cirrhosis at HCC diagnosis	(+)	(–)	(+)	(–)	(+)	(–)	(+)
Labo data at HCC diagnosis							
AST/ALT (IU/ml)	59/30	81/49	27/40	46/25	74/56	25/36	187/90
Treatments	TACE	Surgery TACE	TACE	TACE	TACE/RFA	TACE	TACE/PEIT
Survival (survival' death)	Death	Survival	Death	Death	Survival	Survival	Death
Cause of death	HCC		HCC	Interstitial pneumonia			Ruptured oesophageal varices
Survial time from HCC diagnosis (years)	2.0 year (death)	6.1 year (alive)	1.4 year (death)	6.4 year (death)	4.5 year (alive)	40 year (alive)	3.0 year (death)

HCC, hepatocellular carcinoma; PEIT, precutaneous ethanol injection therapy; RA, rheumatoid arthritis; RFA, radio frequency ablation; SLE, systemic lupus erythematosus; TACE, transcatheter arterial chemoembolization; UDCA, ursodeoxycholic acid.

Table 4. Baseline characteristics of AIH patients with or without HCC

	HCC (+) $n = 7$	HCC (−) $n = 186$	P
Mean age			
Age ≥ 60year	5	93	0.235
Age<60year	2	93	
Gender (male female)	215	14/172	0.106
Mean age at presentation (years)	62.0 ± 10.5 (43–73)	56.4 ± 14.0 (16–84)	0.321
Other autoimmune diseases	3 (42.9%)	48 (25.8%)	0.271
Mean follow-up (years)	12.9 ± 4.8 (7–18)	7.9 ± 4.4 (0.1–21)	0.011
Baseline laboratory values			
AST(<40IUL)	114.00 ± 54.59 (57–224)	402.44 ± 455.67 (29–2718)	0.050
ALT (<40IU/L)	100.29 ± 53.65 (44–185)	420.15 ± 424.62 (18–2020)	0.014*
ALP (<112IU/L)	383.43 ± 197.06 (135–679)	455.89 ± 272.53 (112–2135)	0.611
Bilirubin (mg/dl)	0.74 ± 0.38 (0.3–1.5)	4.07 ± 5.73 (0.27–31.8)	0.015*
Albumin (3.5–5.0g/L)	3.66 ± 0.60 (2.7–4.4)	3.77 ± 0.61 (2.00–5.10)	0.633
IgG (500–1300mg/dl)	2842.43 ± 1008.55 (1480–4280)	2504.64 ± 910.22 (210.2–5211)	0.322
Platelets (15–40×10/μl)	12.80 ± 5.00 × 10^4 (4.40–19.60 × 10^4)	19.51 ± 8.02 × 10^4 (2.00–57.05 × 10^4)	0.015*
ANA+(≥ 1:40)	6/7 (85.7%)	152/181 (84.0%)	0.690
SMA+(≥ 1:40)	0/2	36/86 (41.9%)	0.346
HCV Ab (+)	1 (14.3%)	4 (2.2%)	0.175
Cirrhosis at presentation	5 (71.4%)	16 (8.6%)	<0.001**
Received treatment			
Mean PSL (mg/day)	23.21 ± 15.46 (0–40)	28.92 ± 74.30 (0–1000)	0.991
PSL ≥ 220mg	5 (71.4%)	117 (62.9%)	0.490
PSL aloilff	4 (57.1%)	89 (47.8%)	0.460
PSL+UDCA	2 (28.6%)	39 (21.0%)	0.459
PSL+Aza	0	2 (1.1%)	0.929
UDCA alone	1 (14.3%)	37 (19.9)	0.584
Relapse	4(57.1%)	43 (23.1)	0.061
Liver biopsy specimen available at presentation	(n = 5)	(n = 138)	
Stage of fibrosis			
F0	0	10 (7.2%)	
Fl	1 (20.0%)	34 (24.6%)	
F2	0	35 (25.4%)	
F3	2 (40.0%)	31 (22.5%)	
F4	2 (40.0%)	7 (7.1%)	
ALT	($n = 6$)	($n = 164$)	
1year after first treatment	54.0 ± 27.31 (31–96)	44.7 ± 65.2 (6–521)	0.020*

*P < 0.05; **P < 0.01.

ALP, alkaline phosphate; ALT, alanine aminotransferase; ANA, anti-nuclear antibody; AST, aspartate aminotransferase; Aza, azithioprine; PSL, prednisolone; SMA, anti-smooth muscle antibody; UDCA, ursodeoxy cholic acid.

accession were associated with risk for HCC; male gender ($P = 0.033$) and the presence of cirrhosis ($P = 0.002$) at presentation (Table 5). By multivariate Cox analysis, the presence of cirrhosis (Hazard ratio 11.47, 95% CI 2.13–64.60, $P = 0.005$) was associated independently with risk for HCC (Table 6). These data suggest that male patients and cirrhosis at the onset are at particular risk for HCC.

HCC-free survival rates

Figure 3 presents Kaplan–Meier estimates for the cumulative HCC-free survival rate, based upon the presence or absence of cirrhosis at enrolment. The 15-year survival rate without HCC was 96.6% ±2.7 in AIH patients without cirrhosis, and 62.2% ±13.9 in those with cirrhosis. A log-rank test of the two curves showed a significant difference in that the HCC-free survival rate of AIH patients with cirrhosis was significantly lower than that of those without cirrhosis ($P < 0.0001$).

Discussion

Autoimmune hepatitis is a chronic progressive liver disease caused by immune-mediated destruction of hepatic parenchymal cells. Accurate diagnosis depends on a combination of features scored as recommended by the IAIHG (8), with critical criteria including interface hepatitis histologically, hypergammaglobulinemia and characteristic serum autoantibodies (1). The precise pathogenic processes that lead to AIH are uncertain, but likely depend on a genetic predisposition of the host to

Table 5. Variables associated with increased risk factor for HCC (Univariate Cox proportional hazard model)

Characteristics	Subgroup	HCC		HR (95% CI)	*P* value
		Yes (*n* = 7)	No (*n* = 186)		
Gender	Male	2 (28.6%)	14 (7.5%)	6.058 (1.151–31.869)	0.033
Age	<50	1 (14.3%)	51 (27.4%)	0.325 (0.039–2.707)	0.299
	50–59	1 (14.3%)	42 (22.6%)	0.496 (0.060–4.124)	0.517
	≥60	5 (71.4%)	93 (50.0%)	3.617 (0.699–18.723)	0.125
Other autoimmune disease	(+)	3 (42.9%)	48 (25.8%)	1.730 (0.386–7.750)	0.474
Cirrhosis at presentation	(+)	5 (71.4%)	16 (8.6%)	13.878 (2.670–72.142)	0.002
PSL	(−)	1 (14.3%)	55 (29.6%)	0.330 (0.040–2.748)	0.306
	1–19 mg/day	1 (14.3%)	13 (7.0%)	1.164 (0.138–9.827)	0.889
	20–39 mg/day	4 (57.1%)	60 (32.3%)	3.352 (0.750–14.994)	0.113
	≥40 mg/day	1 (14.3%)	57 (30.6%)	0.484 (0.058–4.053)	0.503
Relapse	(+)	4 (57.1%)	43 (23.1%)	3.789 (0.848–16.936)	0.081

HCC, hepatocellular carcinoma; HR, hazard ratio; PSL, prednisolone.

Table 6. Multivariate analysis of predictive factor for HCC in AIH patients (Cox proportional hazards model)

Variables	*P*	HR (95% CI)
Male gender	0.275	2.572 (0.472–14.008)
Cirrhosis at presentation	0.005	11.741 (2.134–64.602)

CI, confidence interval; HCC, hepatocellular carcinoma; HR, hazard ratio.

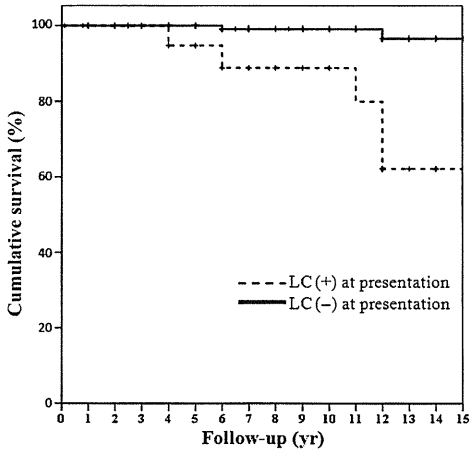

Fig. 3. Fifteen-year HCC-free survival for AIH patients with and without cirrhosis at entry. Kaplan–Meier survival curve comparing HCC-free survival among patients with or without cirrhosis at baseline. The 15-year HCC-free survival was 62.2% for patients with cirrhosis compared with 96.6% for patients without cirrhosis at baseline ($P < 0.0001$).

specific autoimmune reactivity to self-antigens with ensuing hepatic inflammation mediated by T-cell cytotoxicity mechanisms (12, 13).

Our present study has revealed some features of AIH particular to the Japanese population, as well as some differences in practice between Japanese and 'western'-trained internists. In our longitudinal multicentre study on Japanese AIH patients, 71% were treated with corticosteroid during the mean 8-year follow-up period giving an estimated 10-year survival rate as high as 94%. Thus, the survival for corticosteroid-treated type 1 AIH is generally good as in previous reports (3, 4). However, our study did not confirm previous experience that the incidence in AIH of HCC was low, since this did not pertain in patients with histological cirrhosis.

There are a number of reports that patients with chronic viral hepatitis, whether owing to HBV or HCV, are prone to develop HCC in contrast to its infrequency in AIH (7, 14). Park *et al.* (14) reported low incidence of HCC in Caucasian patients with AIH and cirrhosis, observing only one case of HCC among 88 patients with cirrhosis caused by AIH among a total of 212 patients overall, suggesting an incidence of HCC of about 0.1% per patient year (14). In our cohort of 193 patients with AIH, 21 (10.9%) patients suffered from cirrhosis at presentation and, during the follow-up period, seven developed HCC. The patients in our AIH cohort comprised a proportion with cirrhosis similar to that of Park but our follow-up time 8.0 ± 4.5 years was longer. Perhaps the true risk of HCC in AIH patients differs according to ethnicity of the population. Werner *et al.* (6) in the Swedish national-wide AIH cohort showed an overall increase in risk in AIH for all malignancies, mainly contributed to by hepatobiliary cancer. To reiterate, our data indicate that cirrhosis at presentation of AIH was indeed associated with a risk for occurrence of HCC, contrary to traditional belief (15), so that, although HCC occurrence in AIH is lower than that seen in viral-mediated liver disease (15, 16), long-standing cirrhosis may well be a significant risk factor for HCC even in AIH, as described previously (17).

Regarding gender as a risk factor for HCC, Montano-Loza *et al.* (17) reported that male gender and long-standing cirrhosis are combined risk factors for development of HCC in AIH patients. In the present

study, we found a correlation between gender and progression to HCC only in univariate Cox proportional hazard model and not in multivariate Cox proportional hazard model. Thus furthermore large-scale studies are needed in AIH to elucidate the link between gender and HCC.

Although previous concerns have been raised regarding occult viral hepatitis infection as being instrumental in the aetiology of HCC in AIH (18), all patients in our study were negative for serological markers of HBV infection and as well for persisting HCV infection. International diagnostic criteria of AIH (8) allocate negative points for positive HBV or HCV diagnostic tests. Although, seven had anti-HCV antibodies none of these had any evidence of active HCV infection (HCV-RNA negative), and all fulfilled the IAIHG criteria. Whilst seropositivity for HCV was not a statistically significant risk factor for HCC development, the unlikely possibility exists that pre-existing HCV infection was a contributing risk factor for HCC in a small subset of AIH patients. Recently, non-alcoholic steatohepatitis (NASH) has been recognized as an important cause of HCC even in Japan (19). In patients with risk factors for non-alcoholic fatty liver diseases (NAFLD), diagnosis of AIH was confirmed according to the liver histological findings (20). However, we could not rule out the possibility that NASH-related LC was included in our population completely.

In AIH, cirrhosis at presentation is reported to be an important prognostic factor (20, 21). Our study suggests that the long-term outcome for AIH patients with cirrhosis is relatively unfavourable, since the 15-year HCC-free survival rate was only 64% and further that HCC-related death partly contributed to the poorer outcome in AIH with cirrhosis. Consistent with this, Feld *et al.* (22) reported that AIH patients with cirrhosis at presentation had an inferior 10-year survival vs. those without cirrhosis [61.9% vs. 94.0%].

In conclusion, the outcome for Japanese patients with AIH is good, consistent with previous reports. However, 7 (3.6%) of 193 developed HCC and so, among Japanese AIH patients, HCC may not be an uncommon final event in patients with AIH. The presence of cirrhosis at presentation confers an increased risk for future HCC, albeit less commonly than with hepatitis virus-related liver diseases. These risk factors call for a regular screening strategy for HCC in AIH patients with cirrhosis.

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NHO-AIH study group: Kiyoshi Migita, Yuka Jiuchi, Seigo Abiru, Atsumasa Komori, Masahiro Ito, Hiroshi Yatsuhashi, Minoru Nakamura, Hiromi Ishibashi (NHO Nagasaki Medical Center), Yukio Watanabe, Yoko Nakamura (NHO Sagamihara National Hospital), Akira Saito (NHO Nishisaitama-chuo Hospital), Michiyasu Yagura (NHO Tokyo National Hospital), Hajime Ohta (NHO Kanazawa Medical Center), Masaaki Shimada (NHO Nagoya Medical Center), Eiji Mita (NHO Osaka National Hospital), Taizo Hijioka (NHO Osaka Minami Medical Center), Haruhiro Yamashita (NHO Okayama Medical Center), Eiichi Takezaki (NHO Higashi Hiroshima Medical Center), Toyokichi Muro (NHO Oita Medical Center), Hironori Sakai (NHO Beppu Medical Center), Makoto Nakamuta (NHO Kyushu Medical Center).

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PBCの予後予測：自己抗体を基盤に

中村 稔[*,**] 相葉佳洋[*] 小森敦正[*,**]
下田慎治[***] 石橋大海[*,**]

索引用語：原発性胆汁性肝硬変（PBC），長期予後，抗gp210抗体，抗セントロメア抗体，遺伝子多型，バイオマーカー，多施設共同研究

1 はじめに

原発性胆汁性肝硬変（primary biliary cirrhosis, 以下PBCと略す）は肝内小葉間胆管を標的とした臓器特異的自己免疫性疾患と考えられているが，生涯ほとんど進行しない症例から進行して肝移植が必要となる症例まで，種々な重症度の症例が存在する[1~3]．これまでにMayo risk scoreなどの短期予後診断に有用な指標は知られていたが，発症早期において長期予後診断に有用な血清や遺伝子バイオマーカーは知られていなかった．最近，われわれのコホート研究（国立病院機構肝疾患共同研究グループ（NHOSLJ），厚生労働省難治性疾患克服研究事業，難治性の肝・胆道疾患に関する調査研究班，gp210ワーキンググループ）やイタリア，スペイン，米国，カナダ，フランスのコホート研究からPBCの予後予測に有用な指標として，自己抗体（抗gp210抗体，抗セントロメア抗体），病理学的活動性，治療反応性，遺伝子多型などが報告されている[4~14]．これらのPBCの進行に関与するバイオマーカーの同定は，PBCの病態解明や新しい分子標的の同定に繋がる可能性があり，近年，活発に研究が展開されている．

本総説では，自己抗体（抗gp210抗体，抗セントロメア抗体）を基盤としたPBCの長期予後予測について，われわれのcohort studyのデータを中心に紹介する．

2 PBCの病因と臨床像

PBCは病理学的に肝内小葉間胆管の障害・破壊を呈する慢性非化膿性破壊性胆管炎を特徴とする慢性の肝疾患である．中年女性に好発し，初期には無症状のことが多いが，進行

Minoru NAKAMURA et al: Prediction of long-term outcome in PBC based on autoantibody-status
*国立病院機構長崎医療センター臨床研究センター［〒856–8562 長崎県大村市久原 2–1001–1］
**長崎大学大学院医歯薬学総合研究科新興感染症病態制御学系専攻肝臓病学講座
***九州大学大学院病態修復内科学

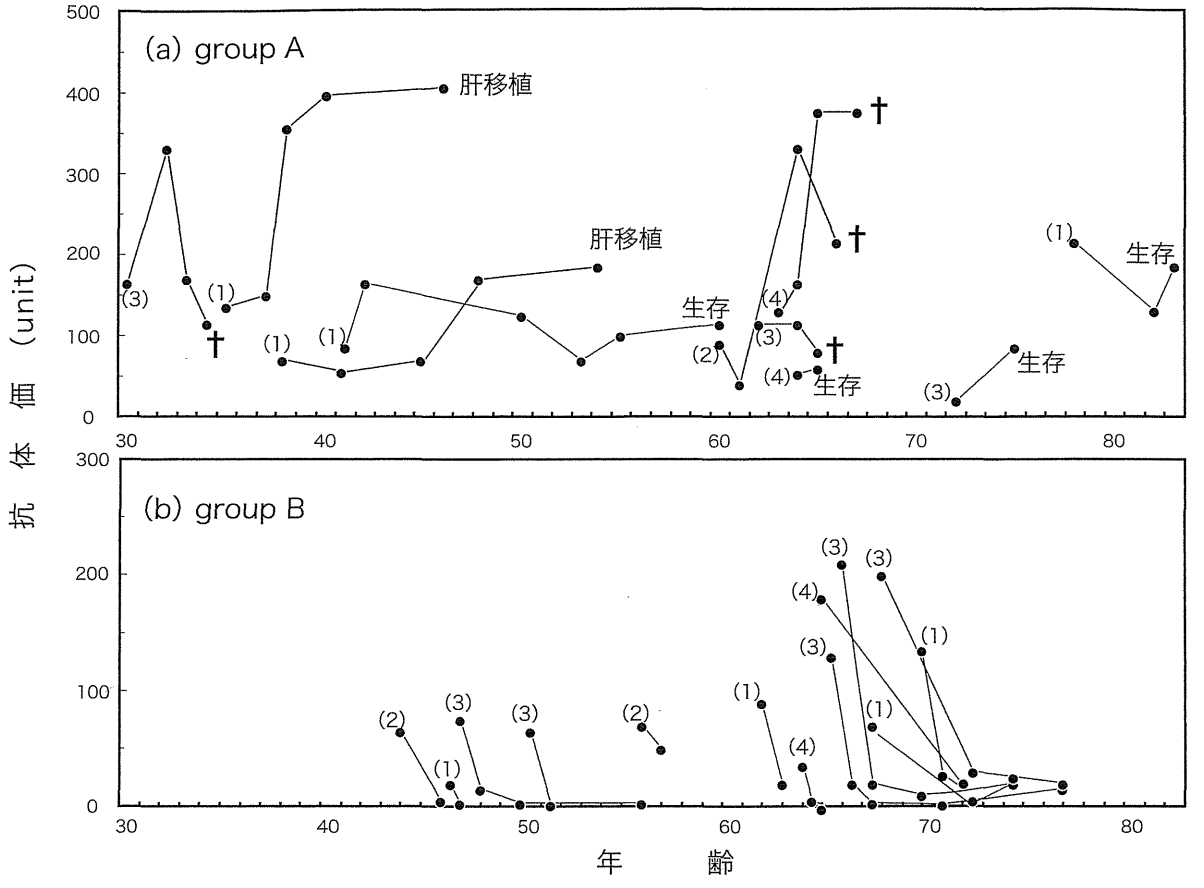

図1　原発性胆汁性肝硬変患者の抗gp210抗体価の経時的変化と転帰(文献4より改変)
抗gp210抗体が持続陽性(group A)の10症例のうち6症例は肝不全死か肝移植に至っていたのに対し,抗gp210抗体がUDCA投与により陰性化した症例(group B)は1例も肝不全や肝移植には至らず全例生存していた.

すると全身の瘙痒感,食道静脈瘤,腹水,黄疸,脳症が出現して肝不全に至り,最終的には肝臓移植以外に救命方法がない.門脈域に小葉間胆管を攻撃しているようにみえるリンパ球の浸潤を認めることや,抗ミトコンドリア抗体(AMA)や抗核抗体などの自己抗体が高率に出現することから小葉間胆管を標的とする自己免疫疾患と考えられているが,その詳細についてはいまだ明らかではない[1~3].近年,診断法の普及により,肝機能異常を契機にAMAを測定されて早期診断される症例や,ウルソデオキシコール酸(ursodeoxycholic acid,以下UDCA)投与により肝機能が正常化し,長期間の経過観察でも病状の進行を認めない無症候性の症例が増加しているが,種々の治療にもかかわらず病状が進行し,肝硬変・肝不全に至る症例がいまだ約10～20%存在すると推定されている.一卵性双生児におけるPBC発症のconcordance rateが60%以上と極めて高いことから,PBC発症には強い遺伝的素因が関係していることも示唆されている[1~3].

3　自己抗体と長期予後

PBCでは,ミトコンドリアや核成分に対するさまざまな自己抗体が出現する.AMAは極めて疾患特異性が高く,PBC患者の90%以上に陽性となるため,PBCの診断には不可欠の検査項目となっている.核膜孔蛋白(gp210)や核小体蛋白(sp100)に対する自

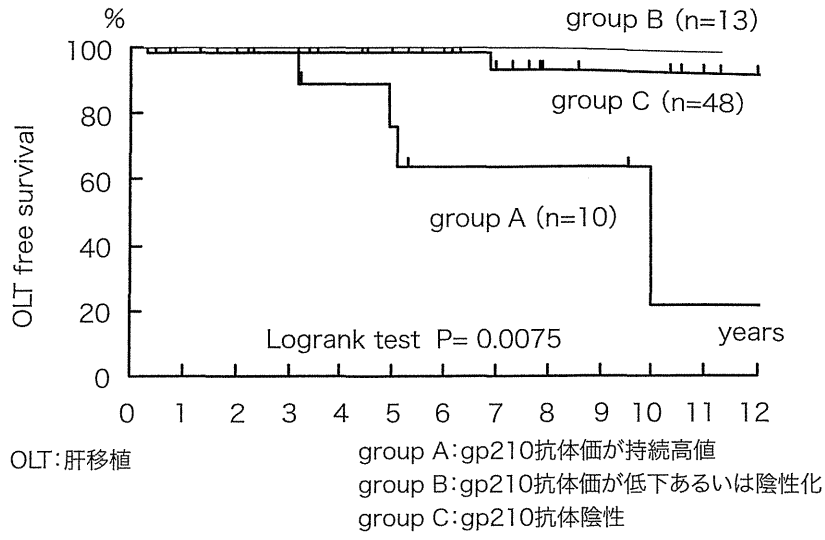

図2　原発性胆汁性肝硬変患者の生存率(文献4より改変)
抗gp210抗体が持続陽性の症例(group A)は，経過中に陰性化した症例(group B)や診断時より抗gp210抗体が陰性であった症例(group C)に比べて有意に肝移植や肝不全死に至る症例の割合が多かった．

己抗体も陽性率は約20～30％と高くはないが，PBC患者に極めて特異的に検出されるため，PBCの診断に有用である．疾患特異性は高くはないが，抗セントロメア抗体も20～40％の症例で陽性となる[15]．

PBCの活動性の評価や予後の診断にgp210やsp100などの核抗原に対する自己抗体が有用であることを示唆する少数の報告はあったが，いずれも横断的研究からの結論であり，PBC症例を長期間観察したコホート研究の報告はなかった[16]．われわれは，AMAの主な標的抗原であるピルビン酸脱水素酵素E2コンポーネント(PDC-E2)とgp210蛋白との間にEIEXDKモチーフを介する分子相同性をみいだしていたことからgp210蛋白に着目し，抗gp210抗体の測定を開始した[17]．

長崎医療センターで過去30年間に保存されていたPBC患者血清の抗gp210抗体価を経時的に測定したところ，抗gp210抗体が持続陽性の10症例のうち6症例は肝不全死か肝移植に至っていたのに対し，抗gp210抗体がUDCA投与により陰性化した症例は1例も肝不全や肝移植には至らず全例生存していた(図1)[4]．また，NHOSLJの71症例の解析から，抗gp210抗体が持続陽性の症例(group A)は，経過中に陰性化した症例(group B)や診断時より抗gp210抗体が陰性であった症例(group C)に比べて有意に肝移植や肝不全死に至る症例の割合が多いことも明らかとなった(図2)，以上から，抗gp210抗体は，PBC患者の長期予後を予測するための有用な血清マーカーであることが示唆された[4]．

その後，NHOSLJに登録された276症例の予後と自己抗体の解析から，抗sp100抗体，抗セントロメア抗体，抗クロマチン抗体の陽性群と陰性群の間には，転帰に有意差を認めなかったが，抗gp210抗体陽性群は陰性群に比べて有意に肝不全死，肝移植に至った症例が多かったことが示された(図3)[5]．また，観察開始時に早期(Scheuer's stage 1, 2)であった217症例について，進行のエンドポイントを胃食道静脈瘤の出現(門脈圧亢進

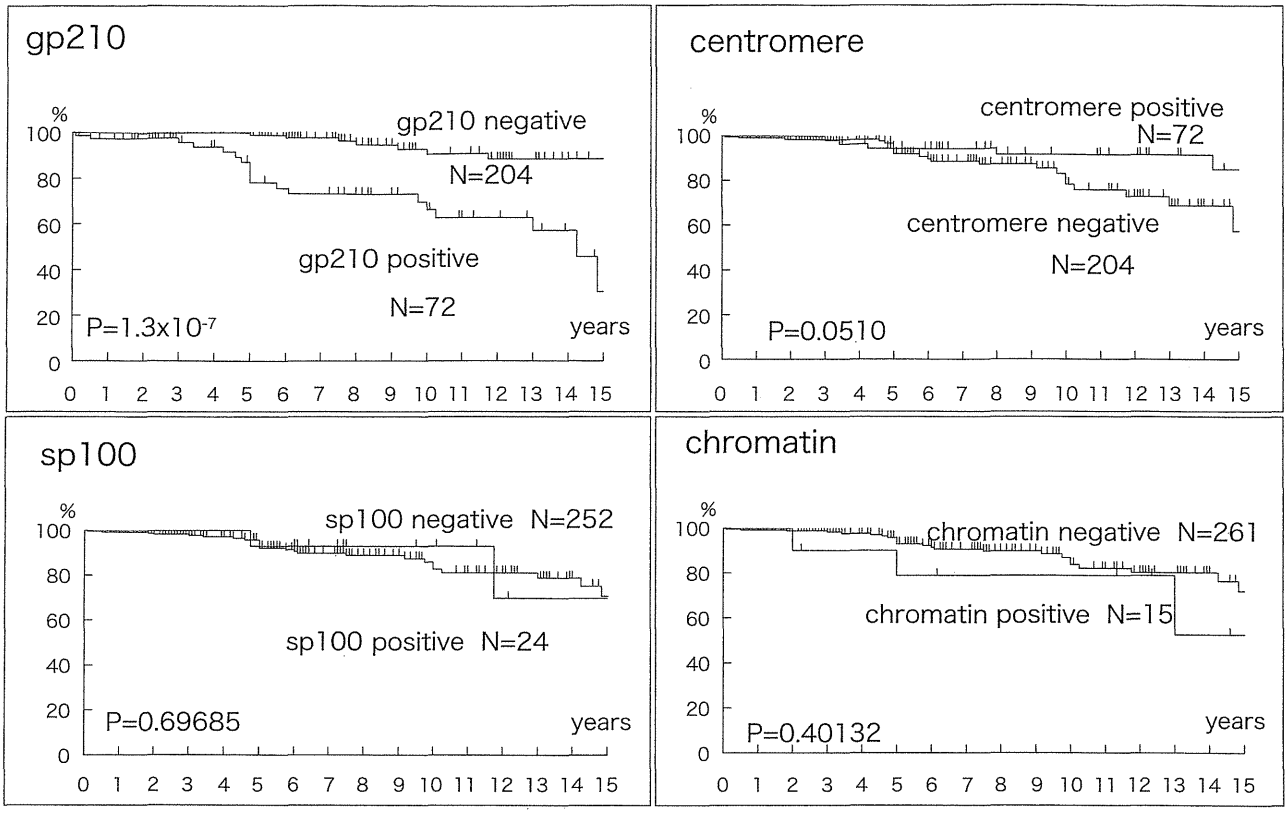

図3　原発性胆汁性肝硬変患者の生存率(文献5より改変)

抗sp100抗体，抗セントロメア抗体，抗クロマチン抗体の陽性群と陰性群の間には，転帰に有意差を認めなかったが，抗gp210抗体陽性群は陰性群に比べて有意に肝不全死，肝移植に至った症例が多かった．

表1　原発性胆汁性肝硬変の進行に対する危険因子 (n=217)

抗gp210抗体陽性は肝不全型(or黄疸型)進行の強い危険因子であり，抗セントロメア抗体陽性は門脈圧亢進症型(or非黄疸型)進行の有意な危険因子であった．

因子	Odds ratio (95% confidence interval)		
	全	門脈圧亢進症 (非黄疸型)	肝不全 (黄疸型)
性　男	—	—	—
年齢(one year $^{-1}$)	—	1.08 (1.01 – 1.16)	—
gp210 抗体陽性	7.09 (2.65 – 20.21)	—	33.78 (5.93 – 636.75)
セントロメア抗体陽性	4.49 (1.66– 12.78)	4.20 (1.31– 14.76)	—
sp100 抗体陽性	—	—	—
クロマチン抗体陽性	—	—	—

(unconditional step-wise logistic regression analysis)

(文献5より改変)

症進行or非黄疸進行)と黄疸・肝不全の出現(肝不全進行or黄疸進行)とに分けて解析したところ，抗gp210抗体陽性は肝不全(or黄疸)進行の強い危険因子であり，抗セントロメア抗体陽性は門脈圧亢進症(or非黄疸)進行の有意な危険因子であった(表1)[5]．以上よりわれわれは，PBCは比較的急速に進行し肝不全，肝移植に至る抗gp210抗体陽性群(肝不全型or黄疸型進行群)と，比較的緩徐に進行し門脈圧亢進症に至る抗セントロメア抗体陽性群(門脈圧亢進症型or非黄疸型進行群)，および長期経過観察でもほとんど進行しない非進行群の3群に分類することを提唱した[3,15]．

次に，これらのNHOSLJのコホート研究の結果を検証する目的で，厚生労働省難治性疾患克服研究事業，難治性の肝・胆道疾患に関する調査研究班に"抗gp210抗体検証ワーキンググループ"が組織され，2007年10月から多施設共同研究が開始されたが，これまでの解析で，①抗gp210抗体が黄疸・肝不全進行の強い危険因子であること，②抗セントロメア抗体が門脈圧亢進症進行の有意な危険因子であることが検証され，③抗gp210抗体陽性症例の病理学的特徴として発症早期より胆管消失や肝炎の所見が高度であることが明らかとなった[18]．

4 遺伝子多型と長期予後

PBC発症には強い遺伝的素因が関係していることが示唆されているため，われわれは，PBCの発症，進行，自己抗体産生に関連する遺伝的素因を同定するために遺伝子多型(一塩基多型：single nucleotide polymorphisms，以下SNPsと略す)の解析を開始しているが，免疫応答や胆汁酸代謝に関連したさまざまな分子が日本人PBCの発症，進行，自己抗体産生に関与していることが明らかとなりつつある．

1. 免疫関連分子

HLA-DRB1遺伝子多型の検討では，HLA-DRB1*0803と*0405はPBC発症の危険因子であるのに対し，HLA-DRB1*1502と*0901は非黄疸進行の危険因子であった．HLA-DRB1*0405と*0803は順に抗gp210抗体産生，抗セントロメア抗体産生の危険因子であった．HLA-DRB1で層別化した解析では，抗gp210抗体はHLA-DRB1の遺伝子多型にかかわらず強い黄疸進行の危険因子であのに対し，抗セントロメア抗体はHLA-DRB1*0405と*0803においてのみ非黄疸進行の有意な危険因子であった．以上より，HLA-DRB1遺伝子多型はPBC発症・進行，抗gp210抗体や抗セントロメア抗体の産生と関連があるだけでなく，これらの抗核抗体のPBCの進行に対する相対危険度を規定していること，すなわちこれら自己抗体を用いたPBCの予後予測には，HLAの遺伝子多型の影響を考慮する必要のあることが明らかとなった[19]．

また，欧米でPBCとの関連が報告されているcytotoxic T-lymphocyte antigen-4 (CTLA4)やsolute carrier family 4 anion exchanger, member 2 (SLC4A2)などの免疫調節に関わる分子の遺伝子多型が，日本人PBCの発症，進行，自己抗体産生に有意に関連することが最近明らかとなっている[13,20]．

2. 胆汁酸代謝関連分子

Multidrug resistance protein 3 (MDR3/ABCB4)は，肝細胞の毛細胆管側に特異的に発現し，リン脂質(フォスファチジルコリン)を胆汁中へ排泄するためのトランスポーター型ATP binding cassette (ABC)蛋白であり，その遺伝子変異により進行性家族性肝内胆汁うっ滞3型 (PFIC3)や妊娠時肝内胆汁うっ滞

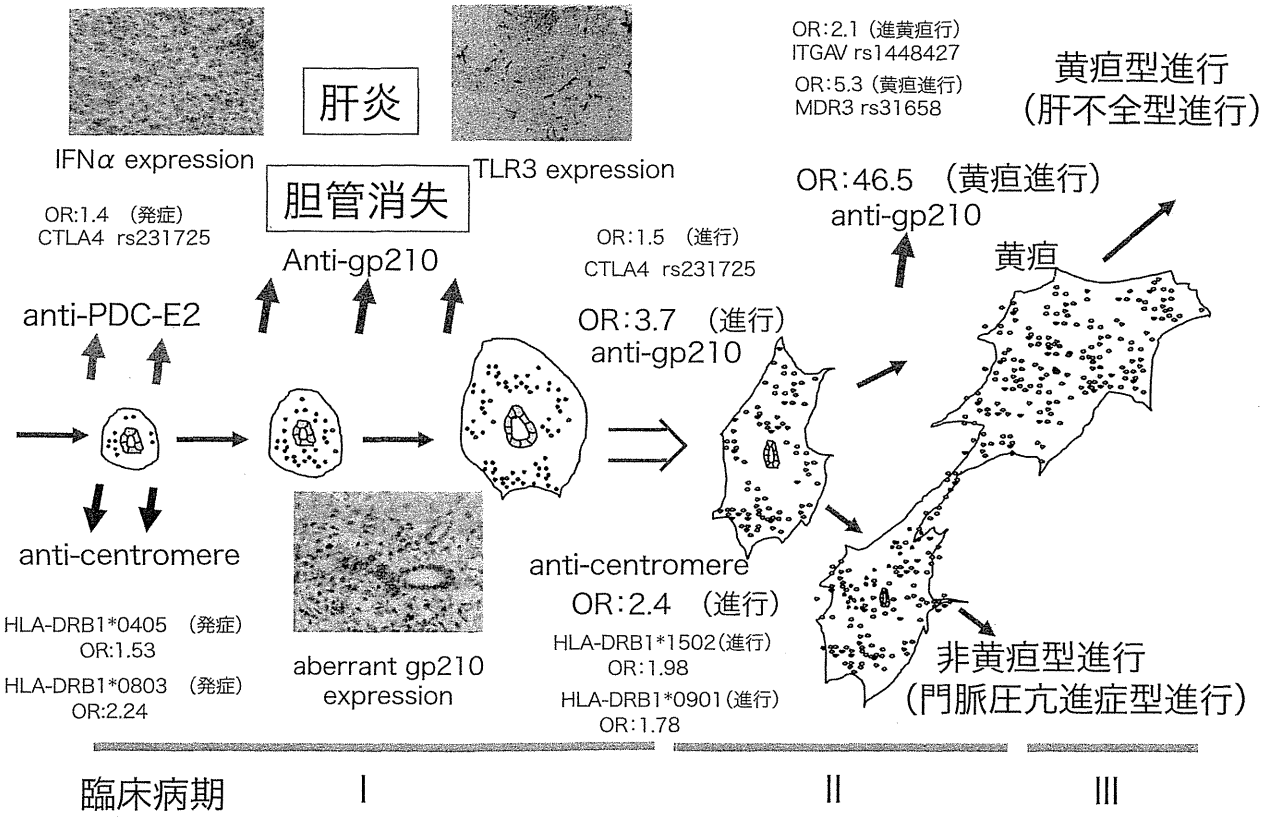

図4 原発性胆汁性肝硬変患者の発症・進行に関与する危険因子(文献4, 5, 12～15, 17～20, 22～24より作成)
今までにわれわれのコホート研究からPBCの発症，進行に有意に関与していることが明らかとなった危険因子を示す．図に示した自己抗体，病理学的活動性，遺伝子多型の他に，図には示していないが治療反応性もPBCの予後に影響する．

症(ICP)が発症することが知られている．われわれは，国際HapMap計画とintegrated Analysis Pipeline(iHap)の遺伝子情報に基づきMDR3遺伝子のSNPsサイトの中から7カ所のTag SNPを選択して解析を行った結果，PBCの進行に有意に関連しているSNPsを3カ所(rs31658, rs31672, rs11492)同定し，その中でrs31658 SNPが黄疸型進行と特に強い相関を示した[12]．リン脂質は胆汁毒性を下げるために重要な胆汁の構成成分であることから，われわれは，rs31658 SNPがMDR3の機能，すなわちリン脂質の排泄能力の個人差を介して，黄疸への進行リスクを規定していると推測している．また，線維化に関係するintegrin aVb6の構成成分であるintegrin aV(ITGAV)のSNPsとPBCの黄疸進行との関連も最近明らかとなった[14]．

5 病理学的活動性，治療反応性と長期予後

PBCの病理学的進行過程がPouponらによって詳細に検討され，胆管障害と肝細胞障害の2つの成分が，主な進行の危険因子であることが示されている[2,8]．われわれは，抗gp210抗体の病理学的特徴を検討し，抗gp210抗体陽性症例は，陰性例に比しinterface hepatitisやlobular inflammationの程度が強いことを報告したが[5]，中沼らが最近提唱している新しい活動度・病期分類[21]を用いて，抗gp210抗体陽性症例は陰性例に比し病初期より肝炎と胆管消失のスコアが高いことが明らかとなった[18]．また，UDCAに