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Running title: Histological staging of PBC

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Abbreviations: AIH, autoimmune hepatitis; ALP, alkaline phosphatase, ALT, alanine aminotransferase; AMA, antimitochondrial antibody; ANA, antinuclear antibodies; AST, aspartate aminotransferase; CNSDC, chronic nonsuppurative destructive cholangitis; γ-GTP, gamma-glutamyl transpeptidase; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; R, risk score; UDCA, ursodeoxycholic acid

Key words: Primary biliary cirrhosis, staging, grading, cholestasis, cholangitis, overlap syndrome

Abstract

Aims: In this study, we attempted to define a new histological staging and grading system to provide more information reflecting the clinical laboratory data and prognosis to hepatologists. *Methods and Results:* First, 17 histological lesions of primary biliary cirrhosis (PBC) were scored in 188 needle liver biopsy specimens. Factor analysis yielded three independent groups of factors: Factor 1 (fibrosis, fibrous piecemeal necrosis, orcein positive granules, bile plugs, Mallory bodies, feathery degeneration, bile duct loss and atypical ductular proliferation); Factor 2 (portal inflammation, eosinophilic infiltration, lymphoid follicle, epithelioid granuloma, interface hepatitis and chronic cholangitis); and Factor 3 (interface hepatitis, lobular hepatitis, acidophilic bodies and pigmented macrophages). Eight findings of Factor 1, but not Factors 2 and 3, were significantly correlated with the clinical laboratory data and scores of Mayo's prognostic model. Factor 1 lesions may reflect the histological progression (staging), while Factor 2 and 3 lesions may relate to necroinflammatory activities (grading). Then, we devised a staging and grading system using three lesions (bile duct loss, fibrosis and orcein positive granules) from Factor 1 and three from Factors 2 and 3 (chronic cholangitis, interface hepatitis and lobular hepatitis). This new system might provide more pathological information of PBC patients to hepatologists.

Introduction

Primary biliary cirrhosis (PBC) is a chronic progressive liver disease of an unknown cause.[1,2] Histologically, the interlobular bile ducts are selectively affected, presenting characteristic findings, such as chronic non-suppurative destructive cholangitis (CNSDC); such affected bile ducts disappear from the liver, and liver cirrhosis and failure finally develop.[3-6] In addition to CNSDC, epithelioid granuloma and/or cholestatic findings in various degrees sometimes contribute to the histological diagnosis of PBC.[7] The average time from diagnosis to end-stage liver disease ranges from several years to 20 years. As for a prognostic indicator, the serum bilirubin concentration is the best among all laboratory values. In addition, several mathematical models based on clinical and laboratory criteria have been proposed to predict disease progression.[8,9]

Histological staging systems of PBC have been proposed by Rubin et al.,[10] Scheuer,[4,11] Popper and Schaffner,[12] and Ludwig et al.[13] In these staging systems, PBC is histologically classified into three or four stages. For example, according to Scheuer's staging, stage 1 is referred to as the florid duct lesion or CNSDC. In stage 2, there is proliferation of the small bile ductules. Stage 3 is characterized by fibrosis or scarring. Stage 4 is cirrhosis. These histological stages reflect the progression of the disease from destruction of the intrahepatic bile duct to fibrosis and cirrhosis. These staging systems were mainly defined based on experiences of established liver pathologists. However, it is

well known that histological changes are heterogeneous in PBC livers and sampling errors are indicated in needle liver biopsies of PBC; histological lesions characterizing different stages could be seen on one liver biopsy and the staging may be different in liver biopsies obtained from different parts of the liver at the same time.[3,4,14] These make the reproducible application of these staging systems difficult in some PBC cases. In addition, pre-existing systems were only for histological staging. Necroinflammatory changes in portal tract or parenchyma differ from case to case. It has not been clarified whether or not necroinflammatory features are related to the clinical characteristics, although the histological degrees of necroinflammation in PBC might be informative in the clinical field, the same as other liver diseases.[15-18] Then, we tried to propose a staging and grading system for PBC using a scoring system of multiple histological features. This system seems to be more objective compared to pre-existing systems because of the scoring system of multiple histological features. In addition, using multiple histological features for staging or grading might contribute to the avoidance of sampling error in the histological evaluation of PBC livers.

Since the publication of the latest staging system, that of Ludwig et al. in 1978,[13] much progress has been made in clinical areas, particularly in therapeutic fields.[19-21] That is, a number of therapies for PBC have been attempted, although most of their efficacies have not been confirmed, except for ursodeoxycholic acid (UDCA) for classical PBC, and

combined UDCA and corticosteroid therapy for overlapping syndrome (PBC plus autoimmune hepatitis).[22,23]

We propose herein a new histological staging and grading system, in which the combination analysis of three items reflects the histological progression toward cirrhosis and also the necroinflammatory activities of primary pathological changes. This new system may be useful in the evaluation of clinical situations of PBC patients.

Patients and Methods

Patient selection and diagnosis

A total of 188 needle liver biopsy specimens from 188 patients who fulfilled the clinical, serological or histological criteria of PBC were evaluated in this study.[1,2] The patients with known causes of liver diseases other than PBC were excluded. These 188 liver biopsy specimens had been prepared consecutively at our university hospital and affiliated hospitals over the past 12 years. The specimens were immediately fixed in formalin and paraffin-embedded, then cut at 3μm thickness for hematoxylin-eosin, reticulin and orcein stainings. The orcein staining was used for the evaluation of the deposition of copper binding proteins in hepatocytes. All of these liver specimens contained at least 5 portal tracts. Histological examinations were performed by two people (KH and YN), evaluating 17 histological features that are elementary and

representative for PBC (Table 1): fibrosis, portal inflammation, eosinophilic infiltration, lymphoid follicle, epithelioid granuloma in the portal tract, interface hepatitis, fibrous piecemeal necrosis, atypical ductular proliferation, orcein-positive granules, bile plugs, Mallory bodies, feathery degeneration, lobular hepatitis, acidophilic bodies, pigmented macrophages in the parenchyma, bile duct loss and chronic cholangitis, including CNSDC. Periportal pericellular fibrosis associated with hepatocellular swelling (cholate stasis) was called "fibrous piecemeal necrosis" in this study.[3,24] Interface hepatitis corresponds to the lymphocytic piecemeal necrosis at the limiting plates.[24] The criteria of scoring of these 17 individual lesions are shown in Table 1.

The clinical data shown in Table 2 were available in all of these patients around the time of liver biopsy. These patients were not under specific therapy, such as UDCA, corticosteroids or D-penicillamine. The patient's prognosis at the time of liver biopsy was predicted using the two previously reported methods, known as the original Mayo model and the updated Mayo model.[8,9] In these models, histological findings are not counted.

In the original Mayo model, a risk score (R) was computed, as follows:

R= 0.871 x log e (bilirubin in mg/dl) -2.53 x log e (albumin in g/dl) + 0.039 x age in years + 2.38 x log e (prothrombin time in seconds) + 0.859 x edema

The edema variable was coded as 0 for no edema and no diuretic therapy, 0.5 for edema present without diuretic therapy or edema resolved with diuretic therapy, and 1 for

edema despite diuretic therapy. Then, S(t) was computed for the patient's probability of survival at least t years as follows:

$$S(t) = S0(t)\exp(R-5.07)$$

S0(t) gives the survival probability for an individual with R= 5.07, which is the mean R from the combined data set of 418 Mayo patients. Using this model, the risk scores (R) of our 188 patients were computed. Furthermore, the risk scores (R) using the updated Mayo model were also computed.

Factor analysis and correlational analysis of 17 histological lesions with clinical laboratory data

First, to identify which histological lesions were related to each other, factor analysis was performed on 17 histological lesions (variables) after scoring. The factor analysis can examine the interrelations among all variables and identify groups of variables whose correlation with each other is greater than the variables in other groups. However, variables in a group were not completely independent from variables in other groups. Then, Spearman's rank-difference correlation coefficient was computed to test the relation between scores of histological findings with respect to factor analysis and clinical laboratory data, including age, alanine and aspartate aminotransferase (ALT and AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (γ-GTP), total bilirubin,

albumin, γ-globulin, total cholesterol, prothrombin time, immunoglobulins (IgG, IgA, IgM), the antimitochondiral antiobodies (AMA) titer, the antinuclear antibodies (ANA) titer, and risk scores of the Mayo model or updated Mayo model. The histological lesion(s) correlated with the risk scores of the Mayo model are available for predicting the prognosis of PBC patients. The statistical study for histological and clinical and laboratory data was performed using software: StatView ver 4.5, Macintosh version (SAS Institute Inc., Cary, North Carolina, USA). They were regarded as significantly correlated when the correlation coefficient was <-0.3 or >0.3 and $p<0.001$.

Application of the new staging and grading system to routine liver specimens of PBC

Three pathologists (YZ, HA and SK) from three different hospitals attempted to apply the new staging and grading system (described in the discussion and result sections) to 36, 41 and 52 needle liver biopsies of PBC from their laboratories (A, B and C), respectively.

Results and Discussion

Factor analysis of 17 histological lesions of PBC livers

As shown in Table 3, factor analysis performed on 17 histological lesions (variables) yielded three independent factors (Factors 1, 2 and 3) under orthogonal (varimax) rotations. The variables, which were loaded at 0.50 or higher on a factor (at least 25% of

variance explained) in each factor are shadowed in Table 3. The percentage of the total variance of Factor 1 was 27.8%, and eight variables on it were loaded at 0.50 or higher. The percentage of the total variance of Factor 2 was 15.9% and six variables were loaded on it at 0.50 or higher. The percentage of the total variance of Factor 3 was 10.2% and four variables were loaded on it at 0.50 or higher. It is likely that the intercorrelation with the variables of the same factor were greater than those with the variables of other factors.

In Factor 1, eight histological lesions (fibrosis, fibrous piecemeal necrosis, orcein positive granules, bile plugs, Mallory bodies, feathery degeneration, bile duct loss and atypical ductular proliferation) were clustered. They seem to be related to the cholestatic findings of the liver, and therefore seem to reflect the histological advancement of PBC.[3-6] Fibrosis usually relates to the progression of chronic liver disease, and may mainly be due to prolonged chronic cholate stasis in the cases of PBC.[3,5,6,21,24] Bile duct loss is the result of chronic destructive cholangitis with biliary epithelial apoptosis and is followed by the development of chronic cholestasis and then fibrosis.[25,26] The other six lesions reflecting long-standing chronic cholestasis may be principally secondary to extensive bile duct loss. Longstanding chronic cholate stasis is generally associated with the subsequent loss of periportal hepatocytes and fibrosis. In this sense, the histological lesions of Factor 1 seem to be secondary lesions due to chronic cholangitis, and it seems reasonable that they are related to each other.

The relationship between the eight histological lesions in Factor 1 and the clinical or laboratory data were confirmed by statistical analysis (Table 4A). That is, all of these histological lesions were correlated with the total bilirubin, and the scores (R) of the original or up-date Mayo models. Especially, orcein positive granules were closely correlated with most clinical and laboratory data, including AST and ALT levels.

Portal inflammation, eosinophilic infiltration, lymph follicle, epithelioid granuloma in the portal tract, interface hepatitis and cholangitis, including CNSDC were clustered in Factor 2; and interface hepatitis, lobular hepatitis, acidophilic bodies and pigmented macrophages were clustered in Factor 3. The lesions clustered in Factor 2 seemed to relate to portal inflammation and cholangitis, including CNSDC, while those in Factor 3 related to parenchymal necroinflammation. Interestingly, no histological lesions clustered in Factors 2 and 3 were correlated with any of the clinical or laboratory data examined (Table 4B, 4C). In addition, these lesions did not correlate to the scores of the two Mayo models. These statistical analyses suggested that Factors 2 and 3 do not reflect the staging of PBC. This may be explainable by the long time span between chronic cholangitis and the histological progression of this disease in the liver and then the prognosis of PBC patients. Interface hepatitis and lobular hepatitis in PBC can be considered to relate to the necroinflammatory activities of PBC, and may not relate directly to the prognosis of PBC patients.[22,27]

While ANA is rather frequently detectable in the sera of PBC patients and a high titer ANA is used as a marker of PBC with evident autoimmune hepatitis (AIH) features,[23,27] it was found in this study that there was no significant correlation between the ANA titer and the liver histology of PBC, bringing into question the immunopathogenetic roles of ANA in PBC.

Proposal of a new histological staging and grading system for PBC

Based on the above-mentioned results and discussion, we propose herein a new histological staging and grading system for PBC.

Staging: The staging is useful for the evaluation of progression toward cirrhosis in any chronic liver disease. It is easy and convenient if only one representative lesion, such as the degree of fibrosis, is applicable to the histological staging of chronic progressive liver disease.[15-17] However, in PBC with well-known notorious histological heterogeneity, more than one essential findings is recommended in combination to achieve a more reliable and objective evaluation of staging.[3,4,21] It was found in this study that all of the eight histological findings clustered in Factor 1 were correlated with the clinical and laboratory data, particularly the prognostic scores of PBC patients. Thus, any of these lesions can be used as the item for the histological staging of PBC.

In this context, we adopted three lesions: fibrosis, bile duct loss and chronic cholestasis (deposition of orcein positive granules). Fibrosis is a common finding in various chronic progressive liver diseases and its degree is, in fact, used for the histological staging of chronic hepatitis and non-alcoholic steatohepatitis (NASH).[15-17] Bile duct loss is rather characteristic to PBC and is the result of immune-mediated biliary damage, and the degree of bile duct loss reflects the subsequent development of cholate stasis (Fig. 1A).[28-30] Among the six cholestatic lesions (fibrous piecemeal necrosis, orcein positive granules, bile plugs, Mallory bodies, feathery degeneration, and atypical ductular proliferation), it was found in this study that orcein positive granules were significantly correlated with most of the clinicopathological data evaluated. In addition, the presence and amount of orcein positive granules are easily evaluable and detectable at relatively early stages of PBC if staining is successful (Fig. 1B), and they become more severe and extensive along the progression of this disease.[31] Furthermore, the incidence of orcein positive granules is higher than those of Mallory bodies, bile plugs and feathery degeneration that become evident only at advanced stages. The criteria for atypical ductular proliferation and fibrous piecemeal necrosis are somewhat subjective, and are not easily evaluated by non-liver histopathologists and clinicians.[4,24] So, we chose orcein positive granules as a representative histological lesion of chronic cholestasis. Thus, three items (fibrosis, bile duct loss and chronic cholestasis [deposition of orcein positive

granules]) constitute the baseline in the new staging system. However, great attention should be drawn to orcein staining. It has been recognized for many years that there are technical problems with orcein staining, depending very much on the synthetic nature of the dye and differing laboratory experience. Because considerable weight is attached to this stain in this system, histological evaluation of orcein staining should be carefully performed in the institutions not familiar with this staining method. Otherwise, the modified staging system without orcein staining (only fibrosis and bile duct loss) might be sufficient in those institutions.

Scores for the degree of the three above-mentioned lesions are shown in Table 5. As for fibrosis (F), stage 0 means that there is almost no fibrosis or fibrosis limited to the portal tracts, stage 1 means fibrosis spreading beyond the portal area with incomplete septal fibrosis, stage 2 means the formation of completely connecting septal fibrosis with variable lobular distortion and stage 3 means cirrhosis (extensive fibrosis with regenerative nodules) (Fig. 1A). As for bile duct loss (B), stage 0 means no bile duct loss in any evaluable portal tracts. In stage 1 and stage 2, bile ducts loss is evident in less than 1/3 of portal tracts and in 1/3 to 2/3 of portal tracts, respectively. In stage 3, the interlobular bile ducts were not seen in any evaluable portal tracts (Fig. 1A). As for chronic cholestasis (C) (orcein positive granules), stage 0 means no deposition in periportal hepatocytes. Stage 1 is characterized by deposition in less than one third of the

periportal hepatocytes of at least one portal tract, and stage 3 by the deposition in more than two thirds of the periportal hepatocytes along all the portal tracts or fibrous septa. Stage 2 is between stage 1 and stage 3 (Fig. 1B).

In addition, three pathologists from different laboratories attempted to evaluate the total number of scores ranging from 0 to 9 in individual cases (Table 6A). Tentatively, we categorized these summed scores into 4 groups; group 1, 0 to 1 (minimal progression); group 2, 2 to 3 (mild progression); group 3, 4 to 5 (moderate progression), and group 4, 6 to 9 (advanced progression). Interestingly, the distribution of PBC cases among the three laboratories was comparable to each other, suggesting that this system, using the total score of three histological items, seems to be applicable clinically.

Grading (activities): The grading is applicable for the evaluation of the inflammatory activities of fundamental lesions of PBC. Ideally, the histological grading of PBC should reflect the fundamental lesions of PBC, and such histological lesions for grading should subside after successful treatment. In this system, we adopted three lesions (chronic cholangitis, including CNSDC, lobular hepatitis and interface hepatitis) for grading. They were chosen from Factors 2 and 3. Chronic cholangitis is known as the fundamental lesion of all PBC cases.[3,10] PBC with evident features of interface hepatitis and lobular hepatitis is known as a hepatitic form of PBC or overlapping syndrome.[3,23,27] In this sense,

interface hepatitis and lobular hepatitis can be used as a factor reflecting the necroinflammatory activities of a hepatitic form of PBC.

Scores for the degree of the three above-mentioned lesions are shown in Table 5. As for chronic cholangitis, grade 0 means absent or ambiguous cholangitis. In grades 1, 2 and 3, evident cholangitis is seen in less than 1/3, 1/3 to 2/3 and more than 2/3 of portal tracts, respectively. As for interface hepatitis, grade 0 means no interface hepatitis, while grades 1, 2 and 3 indicate the presence of interface hepatitis in less than 1/3, 1/3 to 2/3 and more than 2/3 of portal tracts, respectively (Fig. 2A). As for lobular hepatitis, grade 0 means no lobular hepatitis, while grades 1, 2 and 3 are characterized by mild and few focal necroses, multiple focal necrosis and zonal and/or bridging necrosis, respectively (Fig. 2B).

Three pathologists from different laboratories attempted to evaluate this grading system in their cases, and the distribution of these activities is shown in Table 6B. The distributions seem comparable to each other, suggesting that this grading system may be applicable in general hospitals.

We think that this staging and grading system may be useful to give evidence-based pathological information reflecting the clinical features or prognosis to hepatologists. However, prospective studies, such as clinical trials and statistical analyses using this system should be performed in future to verify the precision and relevance of this system.

It might be interesting to examine whether or not our scoring system reflects the kinds of different clinical courses or characteristics of PBC patients, such as rapidly advancing cases, asymptomatic cases, or AMA-negative cases.

In conclusion, we proposed a new staging and grading system for the liver histology of PBC patients that can be applied to routinely stained histological sections, such as with hematoxylin and eosin, orcein, reticulin, or alternative staining for fibrosis. We recommend three items for staging to minimize the sampling errors inherent in PBC liver histology: fibrosis, bile duct loss and chronic cholestasis. We also recommend three histological lesions for the grading (activities) of PBC: chronic cholangitis, interface hepatitis and lobular hepatitis. While it may be a little burdensome for pathologists, we believe this method will provide a more objective evaluation of liver specimens of PBC to clinicians, and also provide new histological data in the study of drug therapy and in other clinical and pathological studies.

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Figure legends

Figure 1. Histopathology of the staging of primary biliary cirrhosis. A: The portal tract is fibrously enlarged and septal fibrosis is incomplete (stage of fibrosis, 1). While the portal vein (p) and hepatic arterial branches (arrow) are recognizable, the bile duct is lost in this portal tract and also in other portal tracts in this biopsy specimen (stage of bile duct loss, 3). H&E stain, x200. B: Deposition of orcein-positive granules (arrows) in the periportal hepatocytes is evident in this and other portal tracts (stage of chronic cholestasis, 3). A,B: Orcein stain, x200, serial sections of the same case.

Figure 2. Histopathology of the grading of primary biliary cirrhosis. A: At the edge of the enlarged portal tract, interface hepatitis is clear in two thirds of the limiting plates (arrows, grade of interface hepatitis, 3). B: Multiple focal necroses are seen in the hepatic parenchyma (arrows, grade of lobular hepatitis, 2). A,B: H&E stain, x100.

Table 1. Evaluation of 17 histological lesions and criteria for their scoring in primary biliary cirrhosis

Histological lesions	Score			
	0	1	2	3
Fibrosis	none or limited in portal tract	periportal fibrosis (incomplete septa)	septal fibrosis (complete septa)	cirrhosis
Portal inflammation	Absent	mild	moderate	severe
Eosinophilic infiltration	Absent	mild	moderate	severe
Lymphoid follicles	Absent	<1/3 of portal tract	1/3-2/3 of portal tract	>2/3 of portal tract
Epithelioid granuloma in portal tract	Absent	<1/3 of portal tract	1/3-2/3 of portal tract	>2/3 of portal tract
Interface hepatitis	Absent	mild	moderate	severe
Fibrous piecemeal necrosis	Absent	mild	moderate	severe
Orcein-positive granules	Absent	<1/3 of periportal area	1/3-2/3 of periportal area	>2/3 of periportal area
Bile plugs	Absent	<1/3 of periportal area	1/3-2/3 of periportal area	>2/3 of periportal area
Mallory bodies	Absent	<1/3 of periportal area	1/3-2/3 of periportal area	>2/3 of periportal area
Feathery degeneration	Absent	<1/3 of periportal area	1/3-2/3 of periportal area	>2/3 of periportal area
Lobular hepatitis	Absent	mild	moderate	severe
Acidophilic bodies	Absent	mild	moderate	severe
Pigmented macrophage	Absent	mild	moderate	severe
Bile duct loss	Absent	<1/3 of portal tract	1/3-2/3 of portal tracts	>2/3 of portal tracts
Cholangitis including CNSDC	Absent	<1/3 of portal tract	1/3-2/3 of portal tracts	>2/3 of portal tracts
Atypical ductular proliferation	Absent	mild	moderate	severe

CNSDC, chronic non-suppurative destructive cholangitis.

Table 2. Main clinical and laboratory data of 188 patients with primary biliary cirrhosis

Patients	Number (%)
Total	188
Female	162 (86.2%)
Male	26 (13.8%)
AMA or AMA-M2	Number (%)
Positive	152 (80.9%)
Negative	36 (19.1%)
Clinical data	Mean ± SD (normal range)
Age (years)	56.5±11.1
AST (IU/L)	74.1±100.7 (9-42)
ALT (IU/L)	74.1±116.8 (2-39)
γ-GTP (IU/L)	230±263.62(8-391)
ALP (IU/L)	502.3±440.3 (86-272)
Total bilirubin (mg/dl)	1.86±4.79 (0.2-1.3)
Total protein (g/dl)	7.57±0.8 (6.6-8.1)
Albumin (g/dl)	4.0±0.6 (3.9-4.9)
Prothrombin time (second)	12.3±2.7 (10.5-11.5)
γ-globulin (g/dl)	1.79±0.75 (0.6-1.3)
IgG (mg/dl)	1972±640.8 (1300-1774)
IgA (mg/dl)	376.4±175.9 (178-355)
IgM (mg/dl)	541.8±309.7 (79-200)
Edema score	0.03±0.18
Original Mayo (R)	4.47±1.49
Up-dated Mayo (R)	4.93±1.83

Table 3. Factor analysis of 17 histological lesions

Histological findings	**Factor 1**	**Factor 2**	**Factor 3**
Fibrosis	0.835	0.006	0.189
Portal inflammation	-0.036	0.817	0.076
Eosinophilic infiltration	-0.249	0.598	0.129
Lymphoid follicles	0.263	0.703	-0.064
Epithelioid granuloma in portal tract	-0.061	0.646	-0.06
Interface hepatitis	0.088	0.574	0.501
Fibrous piecemeal necrosis	0.778	-0.091	-0.034
Orcein-positive granules	0.871	0.026	-0.071
Bile plugs	0.714	-0.122	-0.161
Mallory bodies	0.555	-0.018	-0.103
Feathery degeneration	0.734	-0.095	-0.076
Lobular hepatitis	-0.152	0.323	0.667
Acidophilic bodies	0.09	-0.093	0.772
Pigmented macrophage	0.091	-0.002	0.542
Bile duct loss	0.755	-0.065	0.074
Cholangitis including CNSDC	-0.16	0.831	0.087
Atypical ductular proliferation	0.732	0.069	0.126
% of total variance	27.8	15.9	10.2

CNSDC, chronic non-suppurative destructive cholangitis; shadowed areas, highest loadings for each histological finding >0.50 (>25% of variance explained) are shadowed to facilitate comparison of the factors.

Table 4A. Spearman's rank-difference correlation analysis between scores of each histological lesion of Factor 1 and clinical and laboratory data in primary biliary cirrhosis.

	Factor 1							
	Fibrosis	Bile duct loss	Fibrous piecemeal necrosis	Orcein positive granules	Bile plug	Mallory bodies	Feathery degeneration	Atypical ductular proliferation
Age	0.0092 p.902	-0.0964 p.192	0.0093 p.900	-0.0774 p.360	-0.0422 p.569	0.0942 p.202	-0.0627 p.397	-0.0708 p.338
AST	0.3679 p.000	0.2636 p.001	0.3379 p.000	0.4598 p.000	0.2509 p.002	0.0882 p.277	0.2984 p.000	0.3321 p.000
ALT	0.2717 p.001	0.2041 p.011	0.248 p.002	0.4069 p.000	0.0685 p.400	-0.0222 p.786	0.16 p.048	0.2754 p.001
ALP	0.2872 p.000	0.3359 p.000	0.3167 p.000	0.4156 p.000	0.1389 p.088	0.0608 p.457	0.2677 p.001	0.3066 p.000
γ-GTP	0.2557 p.002	0.3601 p.000	0.3376 p.000	0.4271 p.000	0.0677 p.409	-0.0787 p.337	0.2293 p.005	0.354 p.000
Total protein	-0.0183 p.845	-0.0695 p.457	-0.1816 p.050	-0.056 p.569	-0.267 p.004	-0.1776 p.055	-0.1754 p.059	-0.1799 p.052
Albumin	-0.3981 p.000	-0.2875 p.000	-0.4313 p.000	-0.3974 p.000	-0.3875 p.000	-0.2982 p.000	-0.3806 p.000	-0.3822 p.000
Total cholesterol	0.0175 p.860	0.3296 p.001	0.2648 p.007	0.2152 p.034	-0.0008 p.993	-0.2382 p.015	0.1126 p.255	0.3032 p.002
T-bil	0.381 p.000	0.447 p.000	0.367 p.000	0.57 p.000	0.446 p.000	0.339 p.000	0.456 p.000	0.438 p.000
PT	0.1723 p.018	0.0485 p.509	0.1267 p.083	0.101 p.228	0.3304 p.000	0.32 p.000	0.2379 p.001	0.0288 p.695
γ-globulin	0.221 p.039	0.021 p.847	-0.014 p.894	0.06 p.592	0.072 p.504	0.065 p.545	-0.03 p.781	0.028 p.792
IgG	0.0903 p.331	0.0613 p.510	0.0118 p.899	0.1336 p.166	0.1551 p.093	0.1097 p.237	0.0518 p.577	-0.0462 p.619
IgA	-0.0345 p.715	0.0572 p.546	0.0532 p.574	0.1437 p.144	0.0723 p.445	0.0701 p.459	0.129 p.171	0.0217 p.818
IgM	0.1807 p.037	0.1101 p.207	0.0801 p.359	0.1083 p.231	0.0286 p.744	0.1002 p.251	0.0772 p.377	0.1119 p.200
AMA titer	-0.1595 p.057	-0.1647 p.049	-0.2175 p.009	-0.1826 p.043	-0.1355 p.107	0.016 p.849	-0.1269 p.131	-0.1479 p.078
ANA titer	-0.1464 p.117	-0.1886 p.043	0.0088 p.926	-0.072 p.467	-0.1563 p.094	-0.0573 p.541	-0.1818 p.051	-0.1668 p.073
Original Mayo (R)	0.4138 p.000	0.3965 p.000	0.4464 p.000	0.4278 p.000	0.4447 p.000	0.3447 p.000	0.4524 p.000	0.379 p.000
Up-dated Mayo (R)	0.4136 p.000	0.4045 p.000	0.446 p.000	0.4352 p.000	0.445 p.000	0.3451 p.000	0.454 p.000	0.3851 p.000

Shadow areas, these coefficients were regarded as significantly correlated (shadowed) when the correlation coefficients (upper lane) is in -0.3> or 0.3< and P.<.001.(lower lane) AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, gamma glutamyl transpeptidase; T-bil, total bilirubin; AMA, antimitochondrial antibodies; ANA, antinuclear antibodies.

Table 4B. Spearman's rank-difference correlation analysis between scores of each histological lesion of Factor 2 and clinical and laboratory data in primary biliary cirrhosis

	Factor 2					
	Portal inflammation	Eosinophilic infiltration	Lymphoid follicle	Granuloma in portal area	Lymphocytic piecemeal necrosis	Cholangitis
Age	0.1475 p.045	-0.0183 p.805	0.1544 p.036	0.0627 p.397	0.0646 p.382	0.1172 p.112
AST	0.0213 p.793	0.0579 p.476	0.1884 p.020	0.0341 p.674	0.2645 p.001	-0.008 p.922
ALT	0.0302 p.711	0.0576 p.479	0.1343 p.099	0.002 p.980	0.2576 p.001	0.0063 p.939
ALP	-0.0453 p.580	-0.0046 p.955	0.0262 p.750	0.1127 p.167	0.1142 p.161	0.0638 p.435
γ-GTP	0.0049 p.953	-0.006 p.942	-0.0408 p.620	0.1394 p.088	0.1634 p.045	0.0332 p.686
Total protein	0.1402 p.132	0.2222 p.016	-0.032 p.733	0.082 p.380	0.2024 p.029	0.0968 p.299
Albumin	0.0753 p.305	0.1925 p.008	-0.0487 p.508	0.1054 p.150	-0.0268 p.715	0.2476 p.001
Total cholesterol	0.0392 p.692	-0.0546 p.582	0.0082 p.935	0.0007 p.995	0.0723 p.466	0.057 p.566
T-bil	-0.86 0.2411	-2.14 0.0035	-0.25 0.737	-0.173 0.018	0.111 0.1293	-0.162 0.0265
PT	-0.0998 p.173	-0.0843 p.205	0.0268 p.715	-0.0835 p.255	0.0097 p.895	-0.0295 p.688
γ-globulin	0.17 p.112	0.135 p.207	0.007 p.952	0.085 p.427	0.233 p.03	0.118 p.273
IgG	0.0919 p.322	0.0885 p.341	0.0596 p.523	-0.0192 p.836	0.1561 p.091	0.0149 p.873
IgA	-0.0493 p.603	-0.0199 p.834	-0.0165 p.863	-0.0847 p.370	-0.0381 p.687	-0.0809 p.392
IgM	0.0712 p.415	0.1683 p.053	0.0408 p.642	-0.0206 p.814	0.205 p.018	0.0491 p.575
AMA titer	-0.0022 p.979	0.0693 p.411	-0.0863 p.307	0.0242 p.774	-0.082 p.330	0.0224 p.791
ANA titer	-0.1243 p.184	0.098 p.295	0.0015 p.987	0.0894 p.340	-0.0721 p.442	0.1429 p.126
Original Mayo(R)	-0.0097 p.894	-0.1883 p.010	0.103 p.161	-0.0823 p.261	0.1491 p.041	-0.1356 p.064
Up-dated Mayo(R)	-0.0094 p.898	-0.1923 p.008	0.096 p.191	-0.0856 p.243	0.1503 p.040	-0.1379 p.059

Abbreviations are same as those in Table 4A

Table 4C. Spearman's rank-difference correlation analysis between scores of each histological lesion of Factor 3 and clinical and laboratory data in primary biliary cirrhosis

	Factor 3		
	Lobular hepatitis	Acidophilic body	Pigmented macrophage
Age	0.0234 p.751	-0.0732 p.324	-0.1041 p.159
AST	0.2168 p.007	0.2317 p.004	0.1201 p.139
ALT	0.2519 p.002	0.2787 p.001	0.0914 p.263
ALP	0.0711 p.384	0.0797 p.331	0.0603 p.462
γ-GTP	0.0347 p.673	0.106 p.197	-0.0231 p.779
Total protein	0.2046 p.027	0.0436 p.640	-0.0364 p.698
Albumin	0.1165 p.111	-0.1678 p.022	0.0854 p.245
Total cholesterol	0.0025 p.980	0.0574 p.563	-0.0291 p.769
T-bil	-0.047 0.517	0.022 0.7658	0.142 0.0535
PT	-0.0585 p.425	-0.0547 p.457	0.1536 p.036
γ-globulin	0.176 p.102	0.198 p.065	0.157 p.145
IgG	0.0878 p.344	-0.1059 p.256	-0.0489 p.599
IgA	-0.1007 p.287	-0.0106 p.911	-0.0157 p.868
IgM	0.2074 p.017	0.2371 p.006	0.0334 p.703
AMA titer	0.0186 p.826	-0.0554 p.513	0.0564 p.505
ANA titer	-0.0866 p.355	-0.0649 p.491	0.1063 p.258
Original Mayo(R)	-0.0438 p.551	0.0762 p.300	0.0212 p.774
Up-dated Mayo(R)	-0.0427 p.560	0.0736 p.317	0.0202 p.784

Abbreviations are same as those in Table 4A

Table 5. New histological staging and grading system for the liver or primary biliary cirrhosis

Histological staging	**0**	**1**	**2**	**3**
Fibrosis (F)	absent or limited in portal tracts	periportal fibrosis (incomplete septa)	bridging fibrosis (complete septa) with lobular distortion	cirrhosis (extensive fibrosis and regenerative nodules)
Bile duct loss (B)	absent	<1/3 portal tracts	1/3-2/3 portal tracts	>2/3 portal tracts
Chronic cholestasis (C) (orcein positive granules)	absent	<1/3 periportal areas	1/3-2/3 periportal areas	>2/3 of periportal areas
Histological grading	**0**	**1**	**2**	**3**
Cholangitis (CH)	absent or ambiguous cholangitis	evident cholangitis in <1/3 portal tracts	evident cholangitis in 1/3-2/3 portal tracts	evident cholangitis in >2/3 portal tracts
Interface hepatitis (IH)	absent	focal and mild	moderate	severe and extensive
Lobular hepatitis (LH)	absent	mild and focal	many and multiple, focal necrosis	zonal/bridging necrosis

Table 6A. Distribution of summed score of three histological lesions (fibrosis, bile duct loss and chronic cholestasis*) in individual cases of primary biliary cirrhosis, and arbitrary categorization of summed scores into four groups in three different laboratories

Laboratory A (36†)			Laboratory B (41†)		Laboratory C (52†)	
Summed Score (0 – 9)		Group (1-4)‡	Summed Score (0 – 9)	Group (1-4)‡	Summed Score (0 – 9)	Group (1-4)‡
0	4	11	4	10	0	11
1	7		6		11	
2	2	7	15	11	9	18
3	5		6		9	
4	5	10	7	12	9	13
5	5		5		4	
6	3	8	5	8	5	10
7	1		2		3	
8	2		1		1	
9	2		0		1	

*, deposition of orcein positive granules; †, total number of cases examined in each laboratory; ‡, number of case(s); group 1, summed score of 0-1; group 2, 2-3; group 3, 4-5; and group 4, 6-9.

Table 6B. Distribution of the degree of three histological lesions (cholangitis, interface hepatitis and lobular hepatitis) reflecting the grading (activities) of primary biliary cirrhosis in three different laboratories

	Laboratory A (36†)	Laboratory B (41†)	Laboratory C (52†)
Cholangitis			
0*	4	4	18
1	18	24	23
2	10	13	10
3	4	0	1
Interface hepatitis			
0	3	4	13
1	24	22	28
2	8	14	8
3	1	1	3
Lobular hepatitis			
0	0	5	0
1	26	24	42
2	9	12	9
3	1	0	1

*, 0,1,2,3 is the degree of each lesion (described in the text.); †, total number of the cases examined in each laboratory

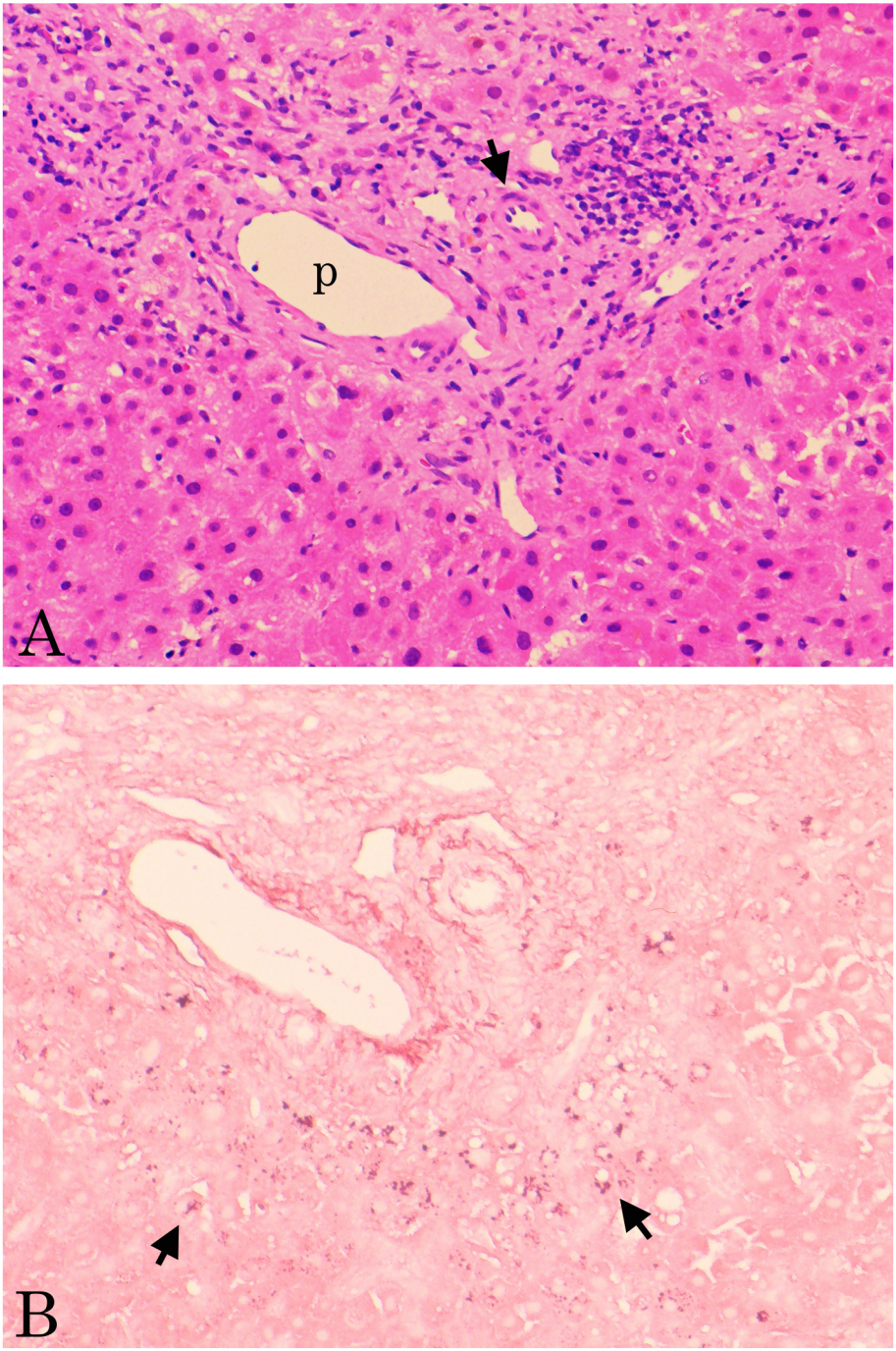

Figure 1. Hiramatsu et al.

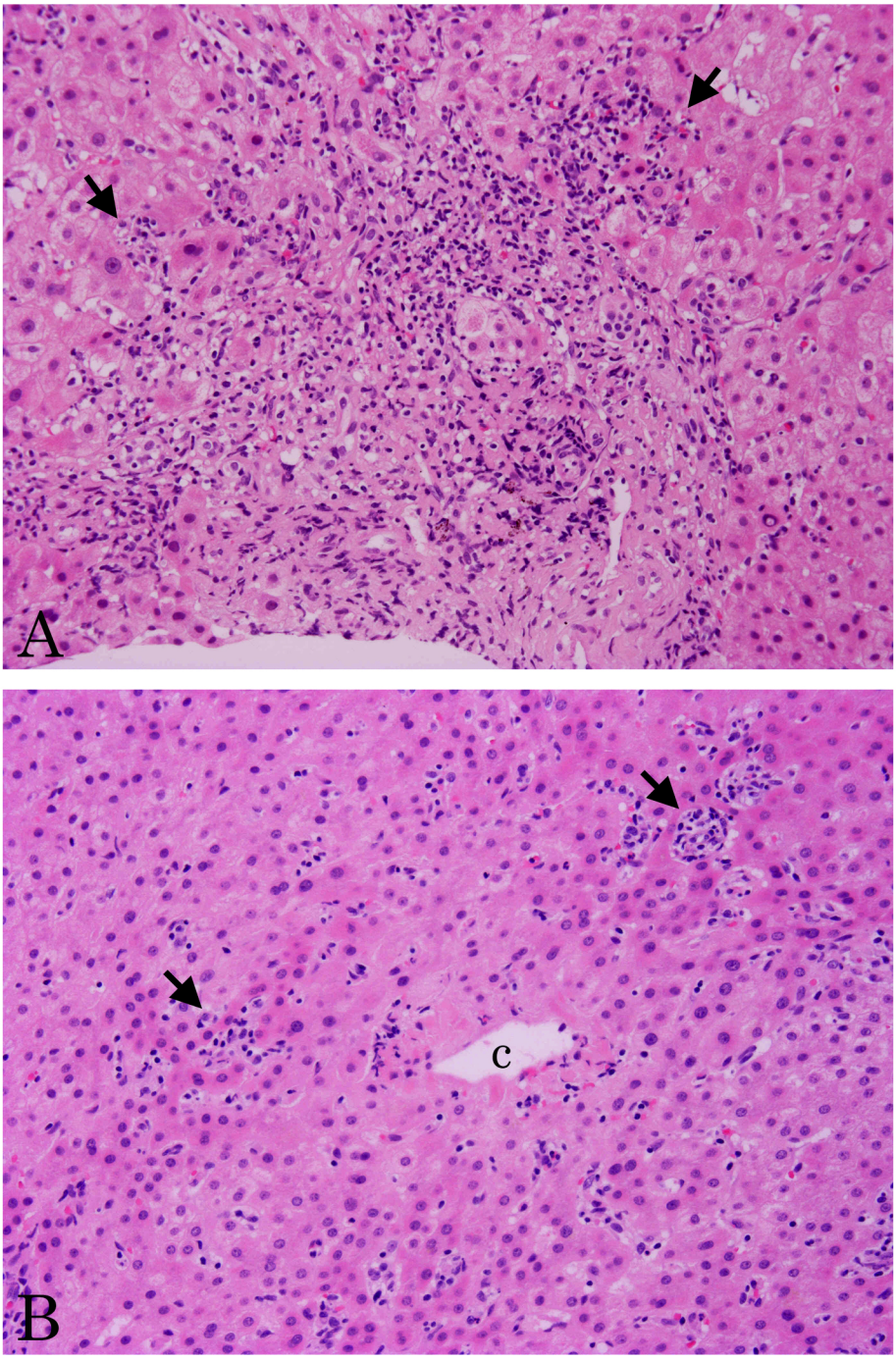

Figure 2. Hiramatsu et al.