Table 3 Medical treatment for patients with primary sclerosing cholangitis (PSC)

Medical treatment	*n*
UDCA monotherapy	89
UDCA+bezafibrate	28
UDCA+PSL	24
UDCA+PSL+bezafibrate	9
PSL mono	7
beza mono	2
(not answered)	38
Total	197
Cases with UDCA	150
Cases with PSL	40
Cases with bezafibrate	39

PSL prednisolone, *UDCA* Ursodeoxycholic acid

Table 4 Endoscopic treatment and efficacy

	PSC ($n = 197$)	IgG4-SC ($n = 43$)
Endoscopic dilatation of bile ducts		
Yes	24 (12%)	4 (9%)
No	157 (80%)	35 (81%)
Unknown/blank	16	4
Endoscopic stenting		
Yes	46 (23%)	15 (35%)
No	110 (56%)	17 (40%)
Unknown/blank	41	11
Effects of endoscopic procedures		
Excellent or fair	39	12
Poor or undetermined	13	2
Unknown/blank	145	29

PSC primary sclerosing cholangitis

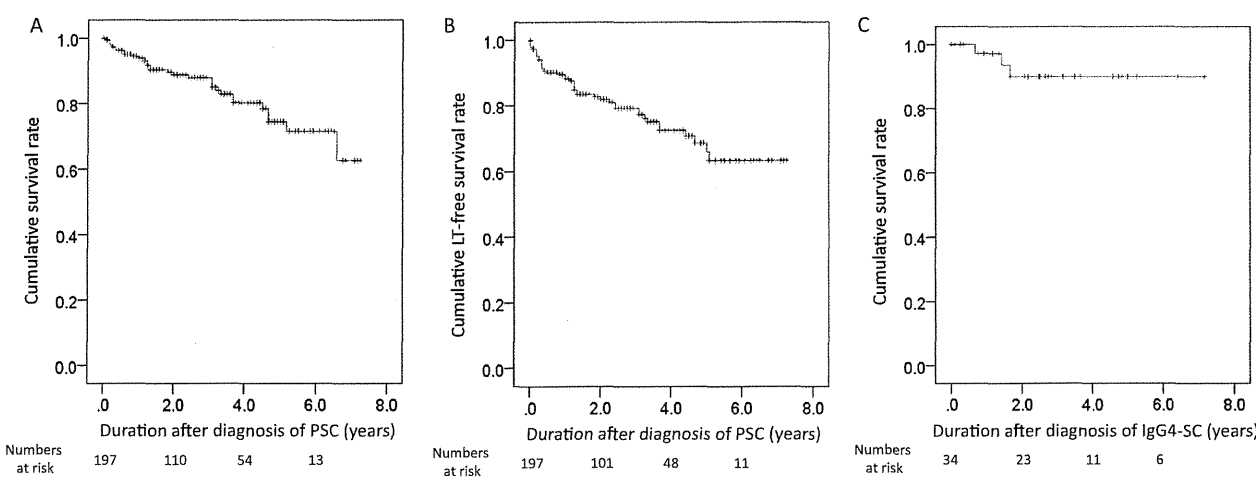

Fig. 7 (**A**) Cumulative survival rate of primary sclerosing cholangitis (PSC). (**B**) Cumulative liver transplantation-free survival rate of PSC. (**C**) Cumulative survival rate of IgG4-SC lacking apparent pancreatic involvement

IgG4-related diseases in other parts; and (4) histopathological findings compatible with IgG4-SC; the effectiveness of corticosteroid therapy is an additional option. In the current survey the differential diagnosis of PSC and IgG4-SC was made using these criteria, and thus the misdiagnosis of IgG4-SC as PSC can theoretically be avoided. Therefore the second peak in the distribution truly reflects the presence of patients who developed PSC in the elderly.

Most previous epidemiological studies from North America and Europe indicated a single age category as the highest risk for developing PSC [6–9], except for a recent study in Canada, which suggested two categories, 18–35 and >65, are higher risk groups than other categories, similar to Japanese studies [18]. It remains obscure why the elderly are at high risk for developing PSC in Japan. One of the reasons is supposed to be the difference of genetic background between Japanese and Caucasians. A questionnaire-based epidemiological study conducted in Japan in 2007 suggested the prevalence of PSC in Japan was 0.95 (95% CI; 0.61–1.29) per 100,000 inhabitants [19], indicating low prevalence rate of PSC in Japan compared to Northern Europe and North America [20]. The genetic diversity among races may affect the prevalence rate as well as the age at risk for development of PSC. Alternatively, another subtype of PSC closely resembling "true" PSC and presenting in the elderly may exist in the Japanese population. However, another possibility that cannot be denied is that IgG4-SC might be still misdiagnosed as PSC even in the current study. This study is a questionnaire-based retrospective design and the diagnosis was made by physicians at each facility. Even using the

common diagnostic criteria of IgG4-SC the diagnosis of difficult cases may vary among facilities, depending on the experiences and knowledge of each physician.

Besides, in this study we confirmed another characteristic in Japanese PSC, already suggested in the previous surveys: the prevalence of IBD as comorbidity appears to be lower in Japanese PSC patients. In various case series from North America and Northern Europe, IBD was a complication in 47–76% of PSC patients [18, 21–27]. However, in Japanese PSC patients, the presence of IBD was restricted to only 37% (125/388) of patients in the 2003 survey [5] and 34% in the current survey. In general, total colonoscopy is frequently performed in Japanese facilities where the diagnosis of PSC is possible with endoscopic retrograde cholangiography; therefore, a lack of a thorough examination of the colon is not a plausible reason for the lower prevalence of IBD. Indeed, total colonoscopy was performed in 53% (206/388) of the PSC patients in our case series [5], suggesting that almost all PSC patients with any bowel symptoms were examined by total colonoscopy. Even after considering that IBD may be present with little or no clinical bowel symptoms in PSC patients, it is unlikely that the prevalence of IBD in Japanese PSC patients could reach 60–80%. Here again, it is unclear why the presence of IBD as comorbidity in patients with PSC is low in Japan compared to those in North America and Europe. Interestingly, another epidemiological study from Asia also demonstrated a lower prevalence of IBD in PSC patients (2/10; 20%) [28], although the number of patients was relatively small. Thus, genetic diversity between Asia and North America/Europe may contribute to the difference of presence of IBD. By contrast, the "contamination" of IgG4-SC may play a role in the low prevalence of IBD as well. The nationwide survey for PSC in Japan repeatedly demonstrated that the age distribution of PSC complicated with IBD demonstrated a single peak in the 20s, while patients with AIP exhibited a small peak in the 60s [4, 5]. Therefore, the prevalence rate of IBD would be increased if misdiagnosed IgG4-SC patients as PSC in the elderly were eliminated from the results.

In conclusion, the third nationwide survey in 2012 has confirmed several interesting clinical details regarding the apparent differences of PSC in Japanese patients and those from Europe/North America; two peaks in the age distribution and lower prevalence of IBD. To investigate whether Japanese PSC is truly characterized by differing clinical features or not, we need to carefully confirm whether PSC patients from the elderly population are "true" PSC patients by central re-examinations of histopathological studies and cholangiographic findings. In addition, it should be validated whether the clinical diagnostic criteria for IgG4-SC as proposed in Japan are appropriate for patients from Europe and North America. Interestingly, the characteristics of IgG4-SC patients observed in the current survey appear to closely resemble those in IgG4-SC patients in the Mayo clinic [29], suggesting that the Japanese diagnostic criteria may be comparable for both populations. Finally, genetic studies to investigate the susceptible genes that contribute to the development of PSC in Japanese patients are needed in order to clarify whether the genetic background of PSC is similar between Japan and Europe/North America.

Acknowledgments This study was conducted and supported by the Intractable Hepato-Biliary Disease Study Group in Japan and the Research Committee to establish diagnostic criteria and development of treatment for systemic IgG4-related sclerosing disease supported by the Research Program of Intractable Disease provided by the Ministry of Health, Labor, and Welfare of Japan.

Conflict of interest None declared.

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Application and Validation of a New Histologic Staging and Grading System for Primary Biliary Cirrhosis

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Background: We proposed a new grading and staging system for primary biliary cirrhosis (PBC), which takes into account the degree of both chronic cholangitis activity (CA) and hepatitic activity (HA) for grading disease activity and that of fibrosis, bile duct loss, and chronic cholestasis for staging. In this study, we validated our new system.

Methods: Using liver biopsy specimens from 166 cases of PBC, chronic cholangitis with mild periductal lymphoplasmacytic infiltration, including chronic nonsuppurative destructive cholangitis, and the combined activity of interface hepatitis and lobular hepatitis were categorized into 4 grades on the basis of their degree and distribution (CA0-3 and HA0-3, respectively). For staging, because orcein staining was not available in this study, 2 criteria (fibrosis and bile duct loss) were independently scored from 0 to 3 on the basis of their degrees, and a final stage score was created from the sum.

Results: Although there was a relatively uniform distribution of CA0/1/2/3 cases, the cases of HA0/1/2/3 were distributed as 21%, 64%, 13%, and 3%, respectively, with a prominent number of cases categorized as having none or mild HA. The distribution of stages 1 to 4 using our system was considerably different from that using the classic system and, importantly, showed a correlation with patient outcome.

Conclusions: Our system revealed that the activities of chronic cholangitis and hepatitis did not correlate with each other in terms of degree and that our staging system properly reflected the outcome of PBC patients. The present study could validate the effectiveness of this new system for characterizing the pathologic condition of PBC.

Key Words: primary biliary cirrhosis, staging, grading

(*J Clin Gastroenterol* 2013;47:174–181)

Primary biliary cirrhosis (PBC) is characterized histologically by progressive loss of intrahepatic small bile ducts, particularly interlobular bile ducts, and serologically by the presence of antimitochondrial antibodies (AMA) and the elevation of serum IgM levels.[1,2] The initial lesion and the histologic hallmark of PBC constitute a distinctive pattern of bile duct injury referred to as chronic nonsuppurative destructive cholangitis (CNSDC). These affected bile ducts eventually disappear and chronic cholestatic features gradually develop.[2] However, hepatitic changes consisting of interface hepatitis and parenchymal necroinflammatory change, which are characteristic in chronic active hepatitis, are also concurrently found in PBC and are closely associated with the disease progression of PBC.[3,4] In recent times, in chronic liver diseases such as chronic viral hepatitis and nonalcoholic steatohepatitis,[5] grading and staging systems have been proposed to objectively assess the degree of disease activity. Because these pathologic evaluating systems are very useful in the estimation of the therapeutic effect and to consider the therapeutic strategy, these systems are now broadly accepted and routinely used. In PBC, bezafibrate and ursodeoxycholic acid have been used to treat biliary damage, and additional treatment with corticosteroid therapy has also been introduced for the overlapping syndrome, the hepatitic form of PBC. Although there has been progress in the development of chemotherapy against PBC, a histologic evaluation of disease activity and therapeutic effect is being subjectively performed, without uniform pathologic evaluating guidelines, such as an applicable grading system for disease activity.

For evaluating the progression of PBC, classic histologic staging systems, such as those proposed by Rubin et al,[6] Scheuer,[7,8] Popper and Schaffner,[9] and Ludwig et al,[10] have been used since the 1960s. However, these classic staging systems do not account for the variable histologic features and/or the differing stages not uncommonly seen in the same liver biopsy specimen or from different regions within the same liver specimen concurrently analyzed.[3,4,11] Further, because histologic findings, particularly cholangiopathy associated with PBC, are heterogenous in a PBC liver, the staging process itself is rather subjective and the possibility of sampling errors should always be considered in needle liver biopsies of PBC. Furthermore, important grading factors, including necroinflammatory changes in bile ducts and HA associated with disease progression, are not fully reflected in these classic classifications. Recently, we proposed a new comprehensive grading and staging system for PBC, which takes into account the multiple histologic findings of chronic cholangitis and HA for grading and those of fibrosis, bile duct loss, and chronic cholestasis for staging.[4] Furthermore, we have proposed a revised, more practical, and convenient version of this new histologic staging and grading system and conducted an interobserver agreement study.[12] In that study, we obtained a "fair agreement" ($\kappa = 0.385$) and concordance rate was 63.9% for staging, although the evaluation of chronic cholangitis and hepatitic change

Received for publication December 21, 2011; accepted August 31, 2012.
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The authors declare that they have nothing to disclose.
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showed "slight agreement" ($\kappa = 0.110$ and 0.197, respectively). In this current study, we validated our new system using liver biopsy specimens of prototypic PBC and compared our analysis using our new classification system with that using the classic classification system.

MATERIALS AND METHODS

Case Selection and Liver Specimens

From clinicopathologic data of autoimmune liver diseases previously collected from 1999 to 2000 by Zeniya et al,[13] 166 cases of PBC were selected as being representative of prototypical PBC. The original diagnoses of these cases were made by on-site physicians using the standard diagnostic criteria proposed by a research group for Japanese intractable liver diseases supported by the Japanese Ministry of Health, Labour, and Welfare,[14] with appropriate clinicopathologic correlation. Patients whose condition meets one of the criteria below are diagnosed as having PBC: (1) CNSDC is histologically observed and laboratory findings do not contradict PBC; (2) AMA and/or antipyruvate dehydrogenase (PDH) is positive. CNSDC is not histologically observed but histologic findings are compatible with PBC; (3) histologic examination was not performed, but either AMA or anti-PDH antibody positivity and clinical findings and course indicate PBC. In this study, liver biopsies were performed in all selected cases before treatments, and the histologic diagnosis of PBC was unanimously confirmed by 3 pathologists in all cases, including AMA or PDH antibody-negative cases. In this cohort, the average age was 56 years (median 52 y), with 23 men and 143 women. Laboratory data were as follows: average serum alanine aminotransferase 64 IU/L (median 50 IU/L), average serum alkaline phosphatase (ALP) ratio (patient's ALP value/upper normal limit of ALP at each respective institution) 1.7 (median 1.3), and average IgM 453 mg/dL. None of the PBC patients had serological markers for HCV or HBV, and 155 patients were serologically positive for AMA by either an immunofluoresence or enzyme-linked immunosorbent assay. Of 23 histologic findings assessed by 3 hepatopathologists in our previous study,[13] portal fibrosis, bile duct injury (including CNSDC and granulomatous cholangitis), bile duct loss, interface hepatitis, and lobular hepatitis were used for our classification system in this study.

Grading System and the New Staging System

The practical and more convenient version of our histologic grading and staging systems, which were modified for needle liver biopsy in our previous study,[12] is shown in Tables 1 and 2. Moreover, the representative histologies defining the grading and staging and the illustrations of Tables 1 and 2 are shown in Figures 1 and 2, respectively. In summary, chronic cholangitis activity (CA) was categorized into 4 grades (CA0-3) on the basis of the degree and distribution of chronic cholangitis. CA0 (no activity) was defined as absent or ambiguous bile duct damage. In CA1 (mild activity), 1 bile duct showed evident chronic cholangitis. In CA2 (moderate activity), 2 or more bile ducts showed evident chronic cholangitis. In CA3 (marked activity), at least 1 damaged bile duct showed CNSDC and/or granulomatous cholangitis. Evident chronic cholangitis was defined as a damaged bile duct surrounded entirely by mild-to-moderate, duct-oriented lymphoplasmacytic inflammation. Hepatitic activity (HA) was also categorized into 4 grades (HA0-3) on the basis of the presence and degree of interface hepatitis and lobular hepatitis. In HA0 (no activity) interface hepatitis was not present. The presence of interface hepatitis affecting at least 10 continuous hepatocytes at the interface of 1 portal tract or fibrous septum was categorized as HA1 (mild activity) and in 2 or more portal tracts or fibrous septa as HA2 (moderate activity). In HA3 (marked activity) interface hepatitis affecting at least 20 continuous hepatocytes at the limiting plate in more than half of the portal tracts or fibrous septa was present throughout the specimen, with entrapment of hepatocytes in the expanded portal tracts. Although no or minimum lobular hepatitis was found in HA0, mild-to-moderate lobular hepatitis was found in HA1 and HA2 and moderate lobular hepatitis in HA3. Occasional zonal necrosis and bridging necrosis were regarded as HA3.

TABLE 1. Grading of Chronic CA and HA in Primary Biliary Cirrhosis

Chronic CA	
CA0 (no activity)	No cholangitis, but mild duct epithelial damage may be present
CA1 (mild activity)	1 bile duct with evident chronic cholangitis
CA2 (moderate activity)	≥ 2 bile ducts with evident chronic cholangitis
CA3 (marked activity)	≥ 1 bile duct with CNSDC
Hepatitic activity (HA)	
HA0 (no activity)	No interface hepatitis and no or minimum lobular hepatitis
HA1 (mild activity)	Interface hepatitis affecting ≥ 10 continuous hepatocytes in 1 portal tract or fibrous septum and mild-to-moderate lobular hepatitis
HA2 (moderate activity)	Interface hepatitis affecting ≥ 10 continuous hepatocytes in ≥ 2 portal tracts or fibrous septa, and mild-to-moderate lobular hepatitis
HA3 (marked activity)	Interface hepatitis affecting ≥ 20 continuous hepatocytes in ≥ 1/2 of portal tracts, and moderate lobular hepatitis or bridging or zonal necrosis

CA indicates cholangitis activity; CNSDC, chronic nonsuppurative destructive cholangitis; HA, hepatitic activity.

In our staging evaluation, because orcein staining was not performed in all cases, we used 2 histologic criteria, fibrosis and bile duct loss. In brief, the fibrosis score was calculated as follows: score 0, almost no fibrosis was present or the fibrosis was confined only to the portal tracts; score 1, fibrosis extended to regions beyond the portal area, occasionally with incomplete septal fibrosis; score 2, completely connecting septal fibrosis or bridging fibrosis with variable lobular distortion; and score 3, established cirrhosis (extensive fibrosis with regenerative nodules). For bile duct loss, interlobular bile ducts were evaluated in well-formed portal tracts with evident hepatic arterial branches and portal vein branches.[2,15] Bile duct loss was scored as follows: score 0, interlobular bile ducts were discernible in all portal tracts; score 1, bile duct loss in less than one third

TABLE 2. Scoring for Stage of Primary Biliary Cirrhosis

Scoring of fibrosis	
Score 0	No portal fibrosis or fibrosis limited to portal tracts
Score 1	Portal fibrosis with periportal fibrosis or incomplete septal fibrosis
Score 2	Bridging fibrosis with variable lobular disarray
Score 3	Liver cirrhosis with regenerative nodules and extensive fibrosis
Scoring of bile duct loss	
Score 0	No bile duct loss
Score 1	Bile duct loss in < 1/3 of portal tracts
Score 2	Bile duct loss in 1/3 to 2/3 of portal tracts
Score 3	Bile duct loss in > 2/3 of portal tracts
Scoring of deposition of orcein-positive granules	
Score 0	No deposition of granules
Score 1	Deposition of granules in a few periportal hepatocytes in < 1/3 of portal tracts
Score 2	Deposition of granules in several periportal hepatocytes in 1/3 to 2/3 of portal tracts
Score 3	Deposition of granules in many hepatocytes in > 2/3 of portal tracts

Staging by sum total of 3 and 2 criteria

Stage	Sum of score: 3 criteria	Sum of score: 2 criteria
Stage 1 (no progression)	0	0
Stage 2 (mild progression)	1-3	1-2
Stage 3 (moderate progression)	4-6	3-4
Stage 4 (advanced progression)	7-9	5-6

3 criteria: fibrosis, bile duct loss, and deposition of orcein-positive granules. 2 criteria: fibrosis and bile duct loss.

of portal tracts; score 2, bile duct loss in one third to two thirds of portal tracts; and score 3, bile duct loss in more than two thirds of portal tracts. The fibrosis and bile duct loss scores were then combined for a total score. The total scores were categorized into the following stages: 0, stage 1 (no progression); 1 to 2, stage 2 (mild progression); 3 to 4, stage 3 (moderate progression); and 5 to 6, stage 4 (advanced progression). For the classic staging system of PBC, the Scheuer system was used for comparison.[7,8]

Prognosis Survey

Approximately 10 years after the collection of biopsies from the cohort, a follow-up survey was conducted in 2010. Of the 166 patients selected in this prototypic PBC cohort, available clinical follow-up data were obtained for 76 patients. This survey was approved by the Ethics Committee of the Jikei University School of Medicine. Survival curves were calculated by the Kaplan-Meier method, and analysis was examined by the log-rank test. A P-value < 0.05 was considered to be statistically significant.

RESULTS

Distribution of the Activity Score (Grading System)

The distribution of CA and HA by our grading system of the 166 PBC cases is shown in Figure 3. The percentage of cases categorized as CA0/1/2/3 was 25%, 23%, 27%, and 25%, respectively, and was relatively uniformly distributed. HA grade peaked in HA1 (mild activity), whereas HA3 (marked activity) was very rare. The distribution of HA0/1/2/3 was 21%, 64%, 13%, and 3%, respectively.

Distribution of Stages and Comparison With the Classic System

The distribution of stages in our staging system using 2 histologic criteria (fibrosis and bile duct loss) and in the classic system by Scheuer[7,8] is shown in Figure 4. In our system, stages 1, 2, 3, and 4 were distributed as 21%, 45%, 21%, and 13%, respectively, with stage 2 (mild progression) being the most frequent. In comparison with the classic classification, in our system the number of cases in stage 1 was markedly decreased and the number of cases in other stages was mildly increased. In particular, from the 46 stage 1 cases as defined by the classic staging system, 32 cases with no fibrosis (fibrosis score 0) and mild bile duct loss (bile duct loss score 1) and 21 cases with mild fibrosis (fibrosis score 1) and no bile duct loss (bile duct score 0) were estimated as stage 2 (total stage score of 1) in our new staging system. In addition, 10 cases showing noncirrhosis (fibrosis score 2) and marked bile duct loss (bile duct loss score 3) classified as stage 3 according to the classic classification were moved to stage 4 under the new system. Figure 5 shows the distribution of CA and HA in each stage. Cases belonging to stages 1, 2, and 3 were scattered along the entire spectrum of CA and HA grades, whereas all cases in stage 4, except for 1, belonged to CA0 or CA1.

Prognostic Value of the New Staging System

Of the 76 patients with available outcome data, 5 underwent liver transplantation and 3 died from liver failure or hepatocellular carcinoma (Table 3). Five cases were lost to follow-up. The remaining patients were confirmed as alive as of 2010. By Kaplan-Meier estimation, the overall survival curve for the 76 PBC patients is shown in Figure 6. Although the distribution of stages using the classic system could not determine the differences in the survival ratio, the survival curve by our system showed a statistically significant difference in the survival ratio between stages (the log-rank test, $P = 0.02$). The 5-year survival probabilities for stages 1, 2, 3, and 4 patients were 100%, 100%, 100%, and 80%, respectively, and the 10-year survival probabilities were 100%, 92%, 82%, and 53%, respectively.

DISCUSSION

Over the last few decades, the pathogenesis and histogenesis of PBC have been gradually clarified, and there has been growing evidence of the clinical benefits of treatment with UDCA and bezafibrate and its combination with corticosteroid for PBC-autoimmune hepatitis (AIH) overlap syndrome.[16–18] With these recent advances and developments, pathologic assessment for therapeutic effect is greatly needed to guide and evaluate PBC treatment; however, an objective and unified evaluation system with clearly outlined criteria has not been developed yet. Chronic cholangitis, including CNSDC and bile duct loss, is thought to be a histologic hallmark of PBC and useful for the pathologic diagnosis of PBC. However, hepatitic changes, such as interface hepatitis and lobular hepatitis, are also not uncommon in the histology of PBC, suggesting that hepatitic changes may also play a role in the histogenesis and disease progression of PBC.[2,3] Therefore, we have proposed a new grading system in PBC that separately

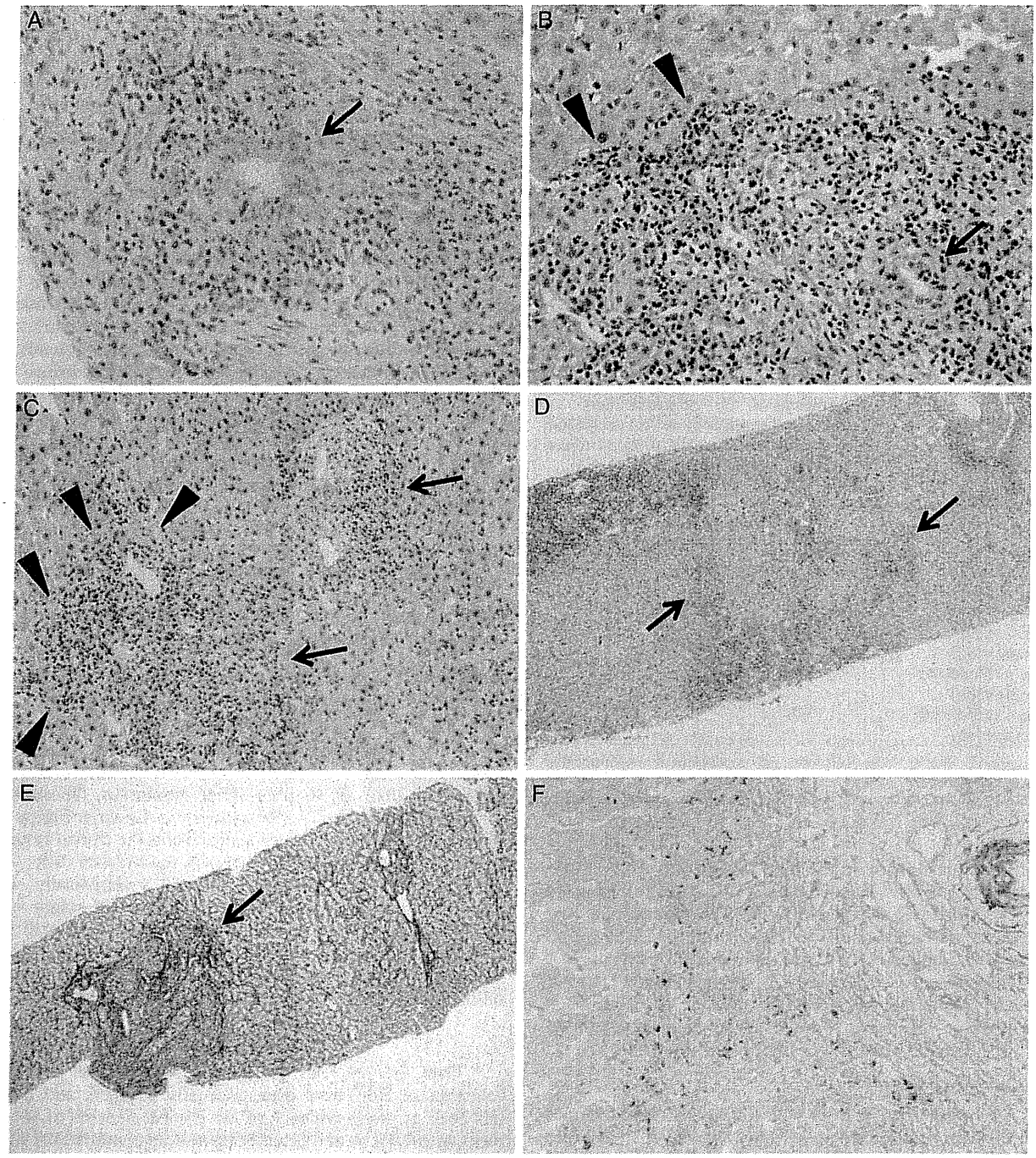

FIGURE 1. Representative findings defining grading and staging of primary biliary cirrhosis. A, Chronic nonsuppurative destructive cholangitis (arrow). Chronic cholangitis activity 3 (CA3). HE staining. B, Evident chronic cholangitis with moderate periductal lymphocytic infiltration (arrow). Part of the limiting plate shows interface hepatitis affecting approximately 10 hepatocytes (arrowheads). HE staining. C, Well-formed interlobular bile ducts are lost in portal tracts (arrows). Interface hepatitis affecting approximately 20 hepatocytes at the interface (arrowheads). Hepatitic activity 3 (HA3). HE staining. D, In parenchyma, moderate lobular hepatitis consisting of several focal necrosis is found (arrows). HE staining. E, Portal tract showing fibrous enlargement without fibrous septa formation (arrow) (score 1 of fibrosis). Reticulin staining. F, Deposition of granules in many hepatocytes is found around enlarged portal tract. Score 3. Orcein staining. HE indicates hematoxylin and eosin.

evaluates chronic CA and HA.[4,12] This independent evaluation is necessary for a more accurate and precise analysis of the pathogenesis of PBC and for the assessment of the effect of various therapies.

In the present study, we evaluated 166 cases of clinicopathologically correlated prototypic PBC using our grading system. After analysis, we showed that the degree of chronic cholangitis (CA0-3) was nearly uniformly

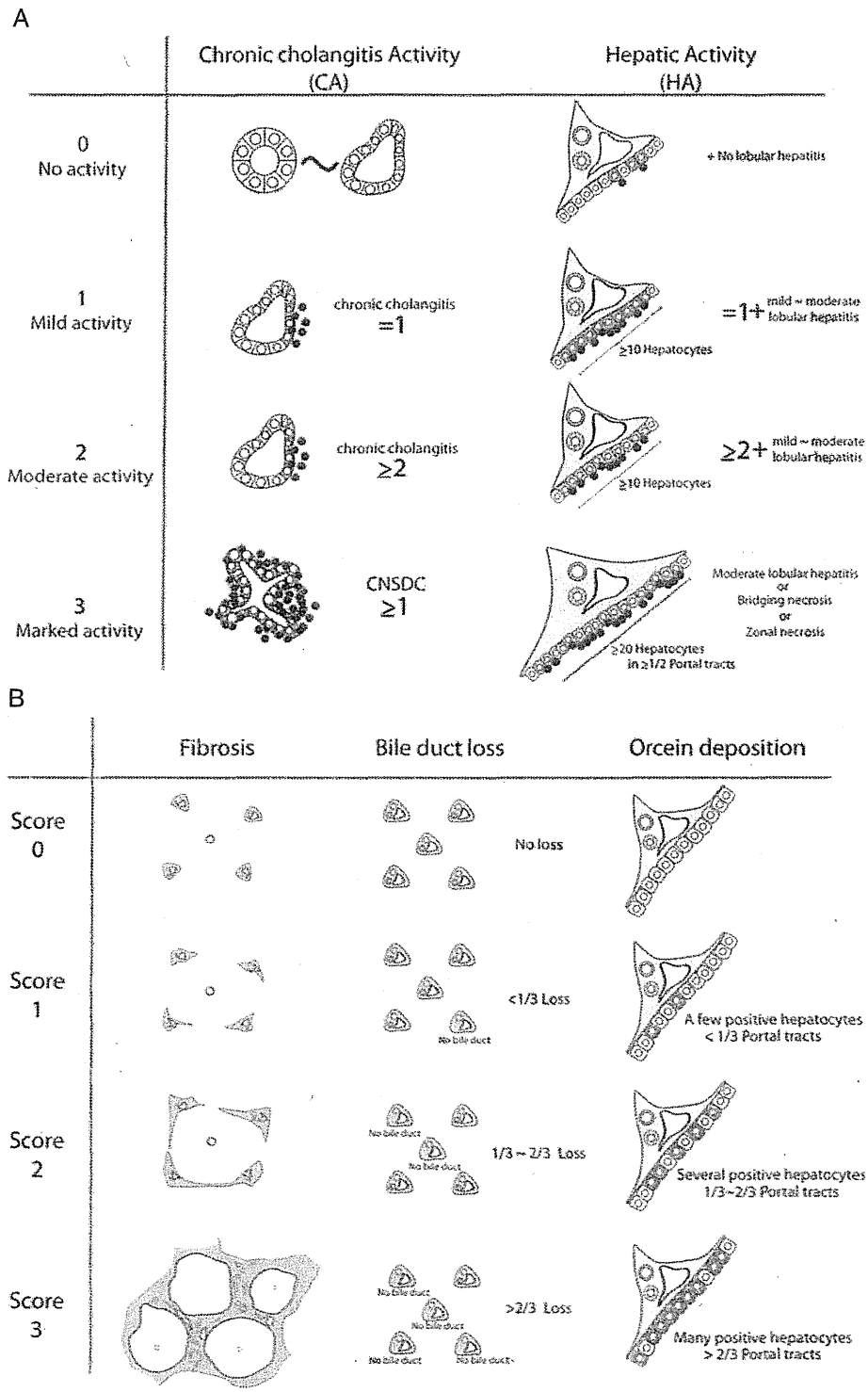

FIGURE 2. Illustration of Tables 1 and 2. A, Grading of chronic cholangitis activity (CA) and hepatitic activity (HA) in primary biliary cirrhosis. B, Scoring for stage of primary biliary cirrhosis. CNSDC indicates chronic nonsuppurative destructive cholangitis.

distributed. Although chronic cholangitis is a distinguishing feature of PBC, this finding suggests that various degrees of chronic cholangitis are found in PBC and up to 25% of PBC cases may not show cholangitis in a given biopsy specimen. However, because grade CA0 was also found in stage 4 cases showing complete bile duct loss (Fig. 5), the significance of evaluating the chronic CA in these types of cases should be deliberated. In contrast to CA, a distribution of HA deviated to the mild category (HA1) in prototypic PBC. In addition to bile duct lesions, HA,

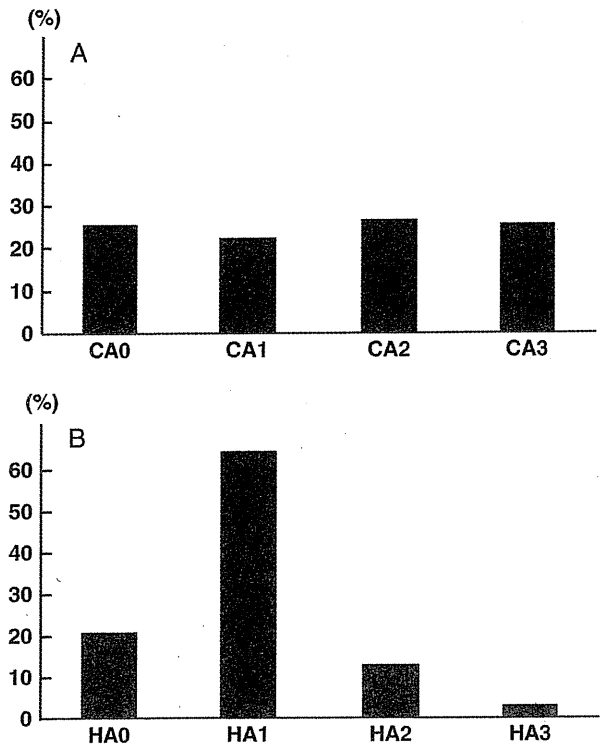

FIGURE 3. The distribution of chronic cholangitis activity (CA) and hepatitis activity (HA) by our new grading system using the prototypic 166 primary biliary cirrhosis cases. A, The percentage of cases categorized as CA0, CA1, CA2, and CA3 was 25%, 23%, 27%, and 25%, respectively. B, The distribution of cases as HA0, HA1, HA2, and HA3 was 21%, 64%, 13%, and 3%, respectively.

consisting of interface hepatitis and/or lobular hepatitis, is concurrently found in PBC, but its degree is usually mild compared with that found in chronic viral hepatitis and AIH. Therefore, the present data, demonstrating that most of the PBC cases were of HA0 or HA1, are in accordance with the typical findings of PBC. Moreover, our results revealed that PBC cases showing marked HA (HA3) only occurred in 3% of PBC cases. PBC and AIH are both

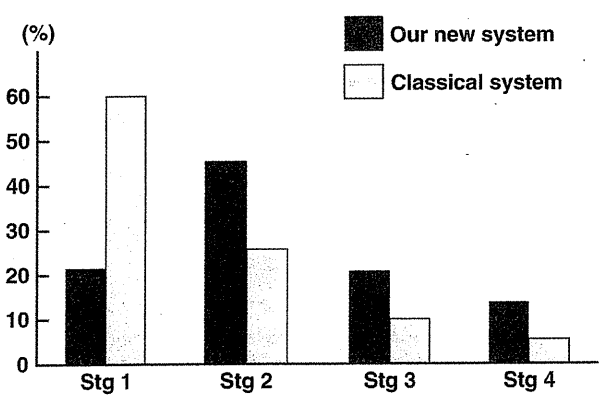

FIGURE 4. The distribution of stages in our new staging systems using 2 histologic criteria (fibrosis and bile duct loss) and in the classic system by Scheuer. In our system, stages 1, 2, 3, and 4 were 21%, 45%, 21%, and 13%, respectively. In contrast, stages 1, 2, 3, and 4 were 59%, 25%, 10%, and 5%, respectively, by the classic system.

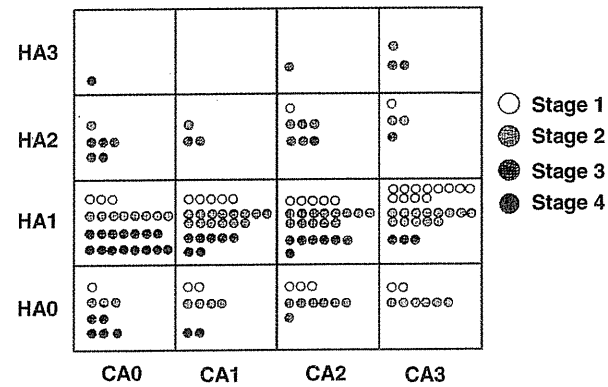

FIGURE 5. Distribution of chronic cholangitis activity (CA) and hepatitis activity (HA) in each stage. Cases of stages 1, 2, and 3 were widely scattered among all grades of CA and HA, but all cases of stage 4, except 1 case (CA2, HA1), belonged to CA0 or CA1.

autoimmune diseases, which selectively affect cholangiocytes and hepatocytes, respectively, but PBC-AIH overlap or the hepatitic form of PBC also has been recognized.[17,18] Because our grading system can independently evaluate cholangitis and hepatitis, this system is very useful in evaluating the differential diagnosis for these diseases, including a possible overlap syndrome, and in guiding therapeutic strategy. For example, in PBC-AIH overlap syndrome, the HA score has been reported to be significantly higher than the CA score.[19]

In the majority of chronic liver disease cases, such as chronic viral hepatitis, the degree of fibrosis constitutes the basis for staging.[5,20,21] However, because cholestasis, in addition to fibrosis, is also a major causative factor of liver failure in PBC,[3,4,22,23] liver transplantation cases of noncirrhotic livers (stage 3 in the classic classification) showing terminal liver failure have often been encountered. Therefore, we have proposed our new staging system, which takes into consideration multiple histologic features: fibrosis, bile duct loss, and, if orcein staining is available, deposition of copper-binding proteins reflecting chronic cholestasis.[4,12] Using these multiple histologic features for staging, we could more precisely define the stage of PBC and reduce sampling errors in the histologic evaluation of PBC. The present study using our system revealed that prototypic PBC cases were distributed as follows: stage 1, 22%; stage 2, 45%; stage 3, 22%; and stage 4, 12%. Stage 2 (mild progression) was the most common. Compared with the classic classification, there was a significant difference in the distribution of stages. Particularly, PBC cases with no or mild fibrosis (fibrosis score 0 or 1) and mild bile duct loss (bile duct score 1) were transferred from stage 1 in the classic system to stage 2 in our new staging system. Further, the possible establishment of a new category of a "no progression state" (stage 1) is unique to our system. Because the number of stage 1 cases in our system was small, the significance of this category should be analyzed. Several concepts such as a presymptomatic state are raised as possibilities, but a more detailed follow-up study is needed to clarify its significance. Moreover, the number of stage 4 cases was increased in our new system, compared with the classic system. The reason for this change is that the noncirrhotic cases (fibrosis score 2) with marked bile duct loss (bile duct score 3) were transferred from stage 3 (noncirrhotic stage) in the classic classification to stage 4 in our new system. Because

TABLE 3. List of Fatal or Liver Transplant Cases

Sex	Age	CA Score	Histologic Staging			Outcome
			HA Score	New Classification (Stage)	Scheuer Classification (Stage)	
Male	73	2	0	2	1	Die from HCC 10 y later (83 y)
Female	36	3	0	2	1	Undergo liver transplantation 6 y later (42 y)
Female	46	2	1	2	1	Undergo liver transplantation 6 y later (52 y)
Female	56	1	1	3	2	Undergo liver transplantation 10 y later (66 y)
Female	34	2	1	3	3	Undergo liver transplantation 6 y later (40 y)
Female	63	2	3	3	3	Die from liver failure 8 y later (71 y)
Female	50	1	1	4	4	Die from liver failure 1 y later (51 y)
Male	39	3	2	4	4	Undergo liver transplantation 7 y later (46 y)

CA indicates cholangitis activity; HA, hepatitic activity; HCC, hepatocellular carcinoma.

these transferred cases already had severe liver dysfunction clinically and the score of bile duct loss correlated with high serum levels of ALP, these cases should be categorized as the terminal stage (stage 4).[23] In contrast to the classic system, the survival curve in our system created a significant difference in survival ratios among the stages, suggesting that our new staging system reflects the progression of PBC patients precisely.

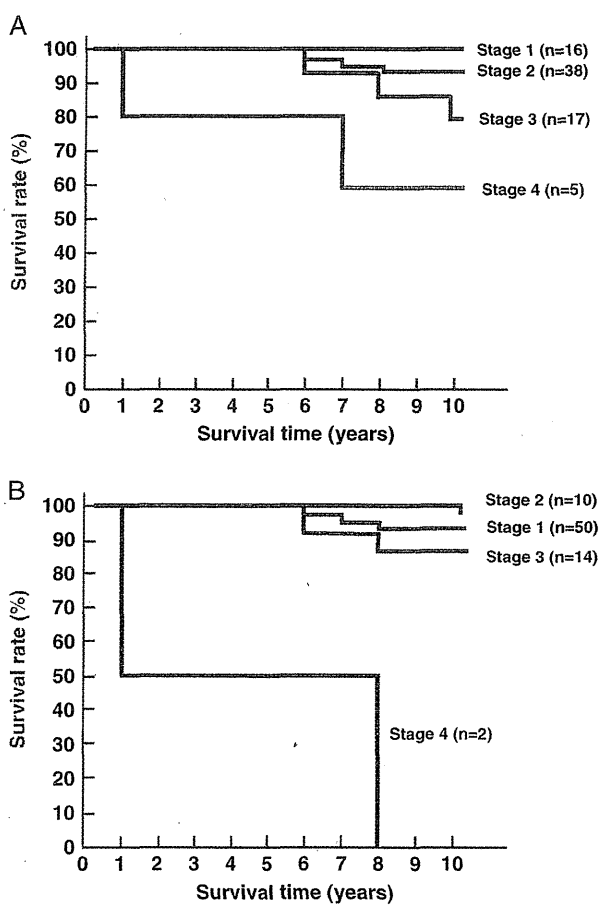

FIGURE 6. The Kaplan-Meier survival analysis based on stages 1 to 4 using the outcome from 76 primary biliary cirrhosis patients. A, The survival curve estimated by our new staging system created a statistically significant difference in survival ratio among stages (the log-rank test, $P=0.02$). B, In the survival curve estimated by the classic system, there was a reversal between stages 1 and stage 2 (the log-rank test, $P=5.87$). Parentheses denote number of cases.

In this study, we evaluated the application and validation of a new histologic staging and grading system using liver biopsy specimens of prototypic PBC patients. Our grading system is unique in that it evaluates separately chronic CA and HA, and our results revealed that the activities of chronic cholangitis and hepatitis do not correlate with respect to degree. The degree of chronic cholangitis (CA0-3) was nearly equally distributed, but the majority of cases had HA0 or HA1 in prototypic PBC. Staging by our new system also differed from the staging by the classic system and, importantly, correlated with the outcome of PBC patients. Our new system is slightly complicated and it may be a little burdensome for pathologists. Moreover, this system does not reflect the distinguishing condition of PBC patients showing marked portal hypertension (nodular regenerative hyperplasia histology) and acute cholestasis, which may reflect a poor outcome. However, we believe that this system will provide more objective information from liver biopsy specimens of PBC to clinicians. In conclusion, our new grading and staging system is a very effective and comprehensive system for evaluating the activity, progression, outcome, and treatment effect in PBC and can be used for studying and diagnosing autoimmune liver diseases including PBC and the PBC/AIH overlap syndrome.

ACKNOWLEDGMENTS

The authors are grateful to members of the study group who participated in this study and to Kumiko Isse, MD (Department of Pathology, Division of Transplantation, University of Pittsburgh Medical Center), for the fine figures.

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Human Pathology (2013) 44, 1107–1117

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Original contribution

Evaluation of a new histologic staging and grading system for primary biliary cirrhosis in comparison with classical systems☆,☆☆

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Received 27 June 2012; revised 22 September 2012; accepted 28 September 2012

Keywords:
Primary biliary cirrhosis;
Grading;
Staging;
Ludwig;
Scheuer

Summary Recently, our research team proposed a new histologic staging and grading system for primary biliary cirrhosis (PBC) that takes into account necroinflammatory activity and histologic heterogeneity. The present study aimed to confirm the usefulness of the new evaluation system. A total of 152 liver biopsy specimens and clinical data (including outcomes in patients with PBC before treatment with ursodeoxycholic acid) were analyzed with respect to the new system. Staging was evaluated on the basis of 3 histologic components (fibrosis, bile duct loss, and deposition of orcein-positive granules), and grading was assessed on the basis of chronic cholangitis activity and hepatitis activity. Concurrently, the classical systems, that is, the Scheuer and Ludwig staging systems, were also assessed and compared with our new system. PBC cases showed different distributions in each stage of the 3 systems. The new staging and grading system reflected liver dysfunctions before specific

Abbreviations: PBC, primary biliary cirrhosis; CA, cholangitis activity; HA, hepatitis activity; CNSDC, chronic nonsuppurative destructive cholangitis; UDCA, ursodeoxycholic acid; Alp, alkaline phosphatase; γ-GTP, γ-glutamyl transpeptidase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; H&E, hematoxylin and eosin; SD, standard deviation; AMA, antimitochondrial antibodies; ANA, antinuclear antibodies; M2Ab, M2 antibodies; HE, hepatic encephalopathy; HCC, hepatocellular carcinoma.

☆ Conflict of interest: The authors declare that they do not have anything to disclose with regard to funding or conflict of interest with respect to this manuscript.

☆☆ Financial support: This work was supported partially by the Research Program of Intractable Disease provided by the Ministry of Health, Labor, and Welfare of Japan and Grants-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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http://dx.doi.org/10.1016/j.humpath.2012.09.017

treatment. This was on a par with the results obtained using the classical systems. Development of cirrhosis-related conditions correlated well with the new staging system compared with the 2 classical staging systems, and in particular, the amount of deposition of orcein-positive granules could reflect development of cirrhosis-related conditions (scores 0-1 versus scores 2-3 groups, $P < .0001$). In conclusion, the new PBC staging system was demonstrated to reflect clinicolaboratory features, and its progression was associated with the development of cirrhosis-related conditions.

1. Introduction

Primary biliary cirrhosis (PBC) is an autoimmune liver disease of unknown cause. Histologically, interlobular bile ducts are selectively affected, presenting chronic nonsuppurative destructive cholangitis (CNSDC). After cholangiopathy, the affected bile ducts finally disappear, causing cholestatic liver failure and cirrhosis [1,2].

Several histologic staging systems for PBC have been proposed since the 1960s, including those by Scheuer [3], Rubin et al [4], and Ludwig et al [5]. In the staging systems devised by Scheuer and Ludwig et al, PBC is classified into 4 stages according to a single histologic feature (eg, CNSDC and fibrosis), similar to the system used for chronic hepatitis. These classical systems have been used widely, but the staging process is subjective. Moreover, the histologic features of PBC are heterogeneously distributed in the entire liver; therefore, sampling errors are often encountered in the same liver biopsy specimens. In the staging system devised by Rubin et al [4], the cirrhotic stage is described clearly but the distinctions among the 3 precirrhotic stages are not.

Recently, we proposed a new grading and staging system for PBC that takes into account the histologic findings of cholangitis and hepatitis activities for grading as well as those of fibrosis, bile duct loss, and chronic cholestasis for staging [6]. Furthermore, we have proposed a revised and more practical version of this new grading and staging system for liver biopsy [7–9]. In the present study, we attempted to evaluate our new staging and grading system by examining relationships with clinicolaboratory features including outcomes in 152 patients with PBC.

2. Materials and methods

2.1. Patient selection and tissue preparations

A total of 152 patients were enrolled in this study. They were histologically diagnosed as having PBC on the basis of liver biopsies (144 needle biopsies and 8 wedge biopsies). All patients received no specific treatments such as ursodeoxycholic acid (UDCA) at the time of biopsy and were serologically negative for hepatitis B surface antigen or hepatitis C antibody. These 152 cases were selected consecutively from the files of Kanazawa University Hospital and Department of Human Pathology, Kanazawa Medical Center, Kanazawa Red Cross Hospital, and Fukui Prefectural Hospital from 1989 to 2011. The 152 cases included 42 cases that had been used in our previous study [6]. Biochemical data (levels of alkaline phosphatase [Alp], γ-glutamyl transpeptidase [γ-GTP], aspartate aminotransferase [AST], alanine aminotransferase [ALT], and total bilirubin) and data on clinical features (gastroesophageal varices assessed by gastrointestinal endoscopy and ascites examined by ultrasonography or computed tomography) were collected for 6 months before biopsy. All liver biopsy specimens were processed routinely, and thin sections were stained with hematoxylin and eosin (H&E), reticulin, Azan-Mallory, and orcein stains. All these liver specimens contained at least 7 portal tracts including 5 complete portal tracts, that is, a portal tract having a portal vein and hepatic arterial branch with or without a bile duct, with an elastic tissue framework confirmed by H&E and orcein staining.

2.2. A new histologic staging and grading system for PBC

Representative histologic findings of PBC as defined by our new histologic staging and grading system are shown in Fig. 1.

2.2.1. Staging

Three lesions (fibrosis, bile duct loss, and deposition of orcein-positive granules) were evaluated for staging. Orcein-positive granules are copper-binding proteins that reflect chronic cholestasis. These 3 items were scored as shown in Table 1. After each of these items was scored, a total was obtained: a total score of 0 was stage 1 (no or minimal progression), 1 to 3 was stage 2 (mild progression), 4 to 6 was stage 3 (moderate progression), and 7 to 9 was stage 4 (advanced progression).

2.2.2. Grading

Chronic cholangitis, including CNSDC, and hepatitis-like changes are representative necroinflammatory lesions of PBC, and they were assessed as shown below and in Table 2.

2.2.2.1. Cholangitis activity. CNSDC was characterized by marked damage to the epithelium of the bile ducts. This was manifested as disarrayed epithelia with swollen or shrunken eosinophils, surrounded entirely by marked duct-

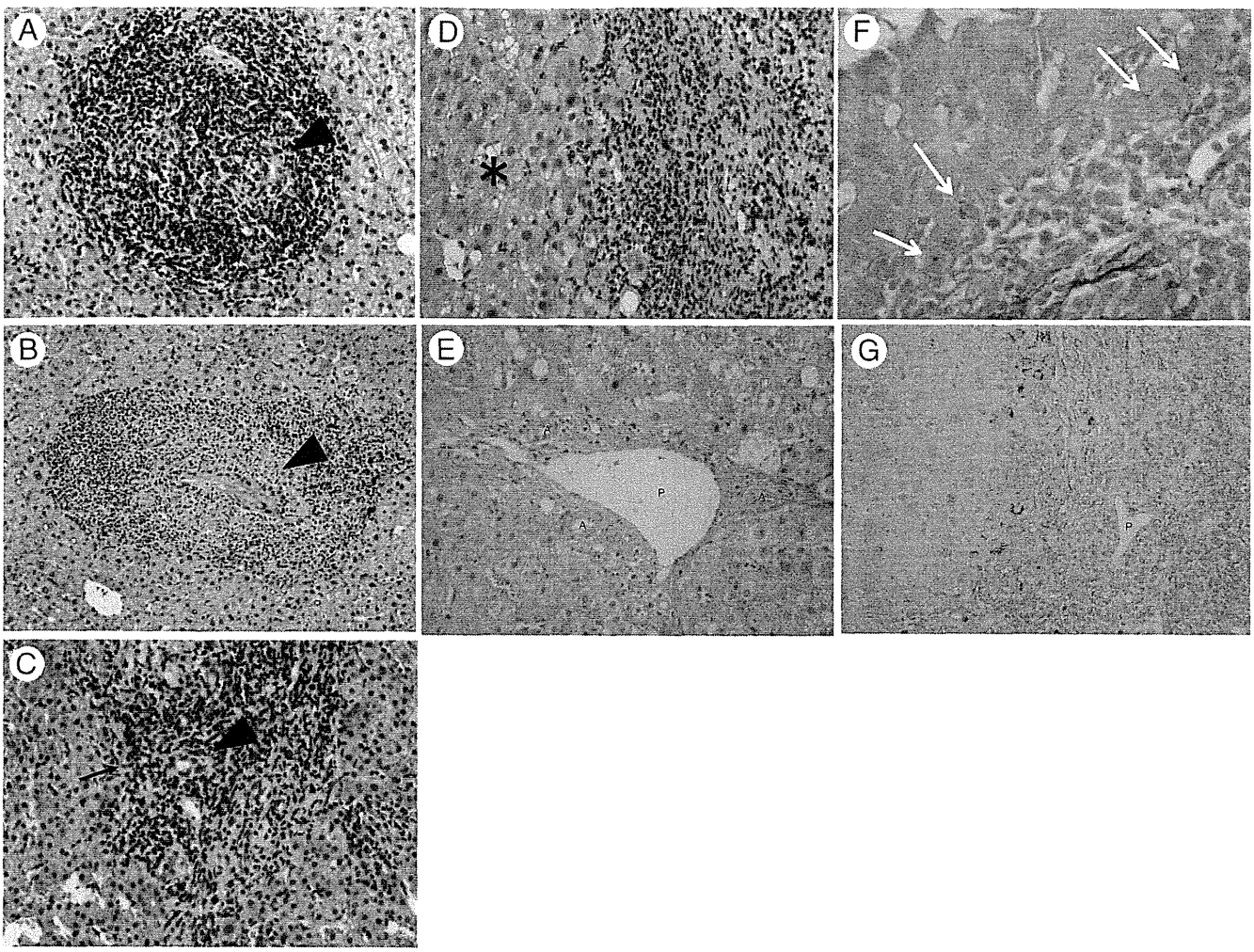

Fig. 1 Histologic findings defining the staging and grading of PBC. A, CNSDC indicates CA3 (arrowhead) (H&E staining, ×400 magnification). B, CA3 includes granulomatous cholangitis (arrowhead) (H&E staining, ×400 magnification). C, Chronic cholangitis (arrowhead). Black arrow denotes interface hepatitis affecting approximately 10 hepatocytes (H&E staining, ×400 magnification). D, Regenerative nodule (*) with interface hepatitis affecting approximately 20 hepatocytes in an HA3 case (H&E staining, ×400 magnification). E, No interlobular bile ducts are found in this portal tract (bile duct loss) (H&E staining, ×400 magnification). F and G, Orcein-positive granules are deposited in zone 1 hepatocytes around 1 portal tract. F, A couple of zone 1 hepatocytes showed orcein-positive granules in their cytoplasm (white arrows). In this case, such deposition was found in zone 1 hepatocytes around less than one-third of portal tracts in the liver biopsy specimen (score 1) (orcein stain, ×1000 magnification). G, Orcein-positive granules are deposited in most zone 1 hepatocytes around 1 portal tract. In this case, such deposition was found in zone 1 hepatocytes around more than two-thirds of portal tracts in the liver biopsy specimen (score 3) (orcein stain, ×400 magnification). Score 2 of deposition of orcein-positive granules represents the amount of deposition in zone 1 hepatocytes in one-third to two-thirds of portal tracts between panels F (score 1) and G (score 3). A, hepatic artery; P, portal vein.

oriented lymphoplasmacytic infiltration and/or periductal epithelioid granulomas (Fig. 1A and B). In contrast, "evident chronic cholangitis" was defined as nonspecific chronic cholangitis surrounded entirely by mild-to-moderate duct-oriented lymphoplasmacytes (Fig. 1C), which is similar to the type of cholangitis encountered occasionally in chronic viral hepatitis [9]. Interlobular bile ducts, which were surrounded by a small number of lymphoplasmacytes or were adjacent to infiltration of lymphoid cells in the portal tract, were not regarded as evident chronic cholangitis. In grade 3, at least 1 damaged bile duct showing CNSDC or a florid duct lesion [3,4] was found.

2.2.2.2. Hepatitis activity. "Interface hepatitis" is defined as lymphocytic interface activity showing damaged hepatocytes with lymphocyte infiltration at the interface of portal tracts or fibrous septa [2,7]. Degree of lobular hepatitis is also taken into account for hepatitis activity (HA) grading.

2.3. Histologic evaluation

Biopsy slides were evaluated by 3 pathologists (Y.K., K.H., and Y.N.) using the new staging and grading system and 2 widely used classical staging systems: the Scheuer system [3] (ie, stage 1: florid duct lesions or CNSDC, stage

Table 1 Scoring for the staging of PBC

Score	Criterion
A. Fibrosis	
0	No portal fibrosis or fibrosis limited to portal tracts
1	Portal fibrosis with periportal fibrosis or incomplete septal fibrosis
2	Bridging fibrosis with variable lobular disarray
3	Liver cirrhosis with regenerative nodules and extensive fibrosis
B. Bile duct loss	
0	No bile duct loss
1	Bile duct loss in less than one-third of portal tracts
2	Bile duct loss in one-third to two-thirds of portal tracts
3	Bile duct loss in more than two-thirds of portal tracts
C. Deposition of orcein-positive granules [a]	
0	No deposition of granules
1	Deposition of granules in a couple of zone 1 hepatocytes at less than one-third of portal tracts
2	Deposition of granules in a variable number of zone 1 hepatocytes at one-third to two-thirds of portal tracts
3	Deposition of granules in most zone 1 hepatocytes at more than two-thirds of portal tracts

[a] See Fig. 1F and G.

Table 2 Grading of the necroinflammatory activity of PBC

Grade	Criteria
A. CA (cholangitis activity)	
0 (no activity)	No cholangitis but mild damage to the epithelium of the duct may be present
1 (mild activity)	1 evident chronic cholangitis in the specimen
2 (moderate activity)	≥2 bile ducts with evident chronic cholangitis
3 (marked activity)	≥1 CNSDC in the specimen
B. HA (hepatitis activity)	
0 (no activity)	No interface hepatitis and no or minimal lobular hepatitis
1 (mild activity)	Interface hepatitis affecting 10 continuous hepatocytes at a limiting plate in 1 portal tract or fibrous septa and mild-to-moderate lobular hepatitis
2 (moderate activity)	Interface hepatitis affecting 10 continuous hepatocytes at limiting plates in ≥2 portal tracts or fibrous septa and mild-to-moderate lobular hepatitis
3 (marked activity)	Interface hepatitis affecting 20 continuous hepatocytes at limiting plates in more than half of the portal tracts and moderate lobular hepatitis or bridging/zonal necrosis

2: proliferation of bile ductules, stage 3: fibrosis or scarring, and stage 4: cirrhosis) and the Ludwig system [5] (ie, stage 1: portal hepatitis, stage 2: periportal hepatitis, stage 3: bridging fibrosis or necrosis, and stage 4: cirrhosis). Grading and staging were evaluated by consensus using a multiheaded microscope and a semiquantitative approach. The HA grade was discrepant in approximately 1 of 5 of cases among the 3 pathologists, but after discussion, a consensus regarding the appropriate grade was reached.

2.4. Definitions of end point

The terminal morphologic feature of PBC is cirrhosis [10]. The number of patients for whom additional follow-up liver biopsies were performed after diagnosis was limited (n = 21). Hence, we set the end point as the occurrence of cirrhosis-related conditions defined by at least one of the following events: histologically proven cirrhosis or cirrhosis-related complications and/or symptoms, that is, ascites, ruptured and/or endoscopically treated gastroesophageal varices, hepatic encephalopathy, hyperbilirubinemia (≥2.0 mg/dL), or hepatocellular carcinoma.

2.5. Statistical analyses

Data are expressed as mean ± SD. Correlations and comparisons of biochemical data of each grade, score, or stage were examined using Spearman correlation coefficient by rank test. Rates of development of cirrhosis-related conditions were estimated using the Kaplan-Meier method and log-rank test. All analyses were 2 sided, and $P < .05$ was considered significant. All statistical analyses were performed using JMP software 8.0 (SAS Institute Inc, Cary, NC).

3. Results

3.1. Distribution of histologic grades, scores, and stages

The main clinical profile and laboratory data of 152 patients with PBC are shown in Table 3. The distribution of grades of cholangitis activity (CA) and HA and scores of the 3 lesions for staging (fibrosis, bile duct loss, and deposition of orcein-positive granules) are shown in Fig. 2A to E. As for necroinflammatory activity, CA3 was the most frequent and CA0, CA1, and CA2 were relatively infrequent. In contrast, HA1 was the most frequent, followed by HA0, HA2, and HA3, indicating that the hepatic form of PBC such as HA2 and HA3 [2,11,12] was the least frequent. With regard to the scores of the 3 lesions, scores 0 and 1 were predominant in each lesion.

The distribution of cases according to the new system and classical 2 staging systems are shown in Fig. 2F to H. Two main findings were noted. First, 95 and 2 of 123 cases of Scheuer stage 1 as well as 20 of 36 cases of Ludwig stage 1 were reclassified as stages 2 and 3 of the new staging system. This was because at least one of the histologic lesions (ie, fibrosis, bile duct loss, or deposition of orcein-positive

Table 3 Clinical characteristics of patients

	Total (n = 152)	Current study only (n = 110) [a]
Age (y), mean ± SD	57 ± 12	56 ± 12
Sex (male/female)	17:135	11:99
Observation period (y), mean ± SD	5.6 ± 5.8 (range, 0-24.8 y)	5.7 ± 6.0 (range, 0-24.8 y)
AMA or M2Ab (+/−)	117:35 (positivity 77.0%)	85:25 (positivity 77.2%)
ANA (+/−)	94:57 (positivity 62.3%) [b]	70:39 (positivity 64.2%)
Symptomatic/asymptomatic	43:108	29:81
UDCA treatment (cases)	136	96
Bezafibrate addition (cases)	26	22
Corticosteroids addition (cases)	19 [c]	14
Laboratory data at the time of biopsy		
Alp (IU/L), mean ± SD	592 ± 501	522 ± 374
γ-GTP (IU/L), mean ± SD	224 ± 203	221 ±207
AST (IU/L), mean ± SD	49.7 ± 31.5	47.6 ± 28.4
ALT (IU/L), mean ± SD	50.6 ± 38.6	50.7 ± 38.3
Total bilirubin (mg/dL), mean ± SD	0.89 ± 1.21	0.76 ± 0.43
Albumin (g/dL), mean ± SD	4.16 ± 0.56 [d]	4.21 ± 0.48
Prothrombin time (s), mean ± SD	11.4 ± 0.81 [d]	11.6 ± 0.61
IgG (mg/dL), mean ± SD	1897 ± 611	1798 ± 536
IgM (mg/dL), mean ± SD	376 ± 267	370 ± 280

Abbreviations: M2Ab, M2 antibodies; ANA, antinuclear antibodies.

[a] Cases that had not been used in our previous study [6].

[b] ANA was available for 151 patients.

[c] Thirteen cases also had corticosteroids for other autoimmune disorders.

[d] Serum albumin concentration and prothrombin time were available for 95 patients (at a single center, Kanazawa University Hospital).

granules) was present in these cases of Scheuer/Ludwig stage 1. Second, 5 of 9 cases classified as stage 4 of the new staging system were reclassified as Scheuer/Ludwig stage 3. This was because these cases showed high scores of bile duct loss and/or deposition of orcein-positive granules but not cirrhosis.

3.2. Relationship between blood biochemistry and the new system and classical staging systems

Herein, we excluded the 42 patients used as cases for the previous study in which this new grading and staging system had been proposed [6]. Therefore, the remaining 110 cases were evaluated. Using Spearman correlation coefficient by rank test, it was found that Alp, γ-GTP, AST, and ALT levels were positively correlated with CA and HA grades; the new staging system and its components (scores of fibrosis, bile duct loss, and deposition of orcein-positive granules); and the 2 classical staging systems (Table 4). Deposition of orcein-positive granules, the new staging system, and the Scheuer staging system also correlated with the total bilirubin level, but the Ludwig staging system did not. Instead, the Ludwig staging system correlated with IgM and IgG levels. HA grade also correlated with the IgG level. Interestingly, antimitochondrial antibody (AMA) and antinuclear antibody titers did not show significant correlation with any staging systems, HA and CA grades, or other histologic lesions for staging.

3.3. Histologic lesions, staging systems, and patient outcome

All patients were followed up for a mean period of 5 years (range, 0-24.8 years). A total of 136 patients were treated with UDCA after liver biopsies (Table 3), whereas 9 patients were followed up (range, 0.5-5 years) without UDCA treatment because of early stage of stable disease. The remaining 7 patients changed hospital immediately after liver biopsy and could not be followed up. A total of 23 patients presented with 1 or several cirrhosis-related conditions (Table 5), and such conditions were already observed in 9 patients at the time of liver biopsy and developed during follow-up in the remaining 14 patients. Therefore, these 9 cases were excluded from the subsequent prognostic studies.

The development of cirrhosis-related conditions at 10 years of follow-up in the 3 systems (Fig. 3) was found in 0%, 12.6%, 40.6%, and 100% of stage 1, 2, 3, and 4 cases of the new system; 7.5%, 100% (at 5 years), and 40.3% of stage 1, 2, and 3 cases of the Scheuer system; and 17.6%, 4.6%, and 47.4% of stages 1, 2, and 3 cases of the Ludwig system. Using the log-rank test, all 3 systems showed significance with the development of these complications and/or symptoms ($P < .01$). Interestingly, the rate of development increased according to the stage progression of the new system, and significant difference was observed between stages 2 and 3 ($P < .01$). Moreover, patients with stage 1 of

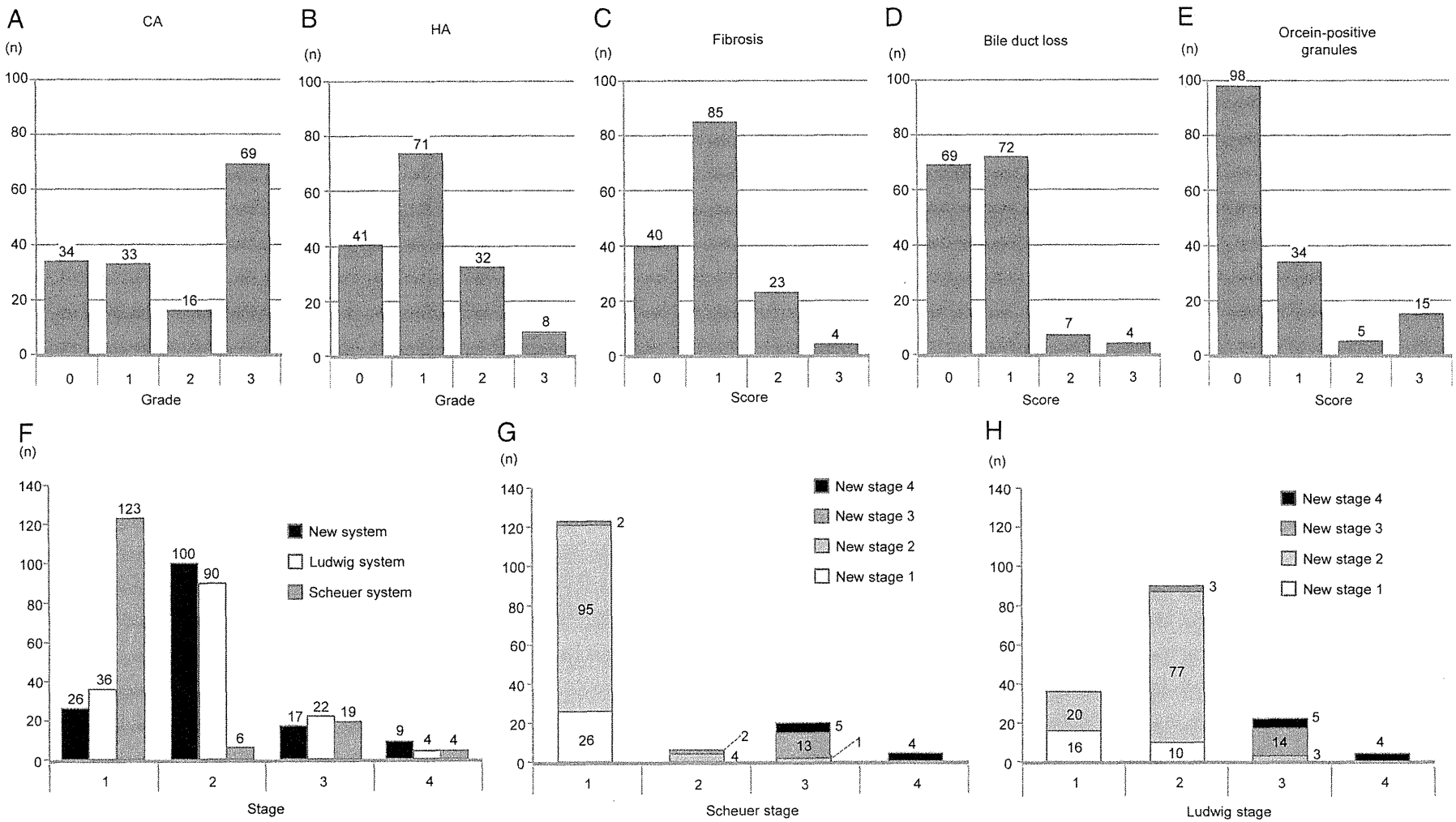

Fig. 2 A-E, Distribution of each grade and score according to the new staging and grading system for PBC. A, CA. B, HA. C, Fibrosis. D, Bile duct loss. E, Deposition of orcein-positive granules. F-H, Comparison of each stage for the new, Scheuer, and Ludwig systems. G and H, Each population was categorized by the new system in each stage according to the Scheuer and Ludwig systems, respectively.

Table 4 Analysis between grading and staging systems and laboratory data for PBC using Spearman correlation coefficient by rank test (n = 110)

	CA	HA	Fibrosis	Bile duct loss	Orcein-positive granules	New system	Scheuer system	Ludwig system
Alp	0.2619*	0.2047*	0.2531*	0.3233*	0.4222*	0.3959*	0.3934*	0.2107*
	$P = .0062$*	$P = .0336$*	$P = .0082$*	$P = .0006$*	$P < .0001$*	$P < .0001$*	$P < .0001$*	$P = .0286$*
γ-GTP	0.2061*	0.2004*	0.3923*	0.3029*	0.4265*	0.4322*	0.4204*	0.2272*
	$P = .0323$*	$P = .0376$*	$P < .0001$*	$P = .0014$*	$P < .0001$*	$P < .0001$*	$P < .0001$*	$P = .0181$*
AST	0.2722*	0.3915*	0.3884*	0.2264*	0.3766*	0.4209*	0.4476*	0.4019*
	$P = .0044$*	$P < .0001$*	$P < .0001$*	$P = .0185$*	$P < .0001$*	$P < .0001$*	$P < .0001$*	$P < .0001$*
ALT	0.2580*	0.3797*	0.3289*	0.1754	0.3238*	0.4363*	0.4139*	0.3804*
	$P = .0070$*	$P < .0001$*	$P = .0005$*	$P = 0.0694$	$P = .0006$*	$P < .0001$*	$P < .0001$*	$P < .0001$*
Total bilirubin	−0.0192	0.0119	0.0815	0.1626	0.2687*	0.2034*	0.2695*	0.0937
	$P = .8443$	$P = .9028$	$P = .4041$	$P = .0943$	$P = .0051$*	$P = .0356$*	$P = .0050$*	$P = .3371$
IgM	0.1156	−0.0104	0.1226	0.1270	0.1590	0.1793	0.1949*	0.1909*
	$P = .2334$	$P = .9146$	$P = .2062$	$P = .1901$	$P = .1003$	$P = .0634$	$P = .0432$*	$P = .0478$*
IgG	−0.0241	0.2122*	0.0889	0.1868	0.0845	0.0782	0.0690	0.2105*
	$P = .8070$	$P = .0297$*	$P = .3674$	$P = .0563$	$P = .3916$	$P = .4278$	$P = .4843$	$P = .0311$*
AMA titer[a]	−0.1887	0.0440	−0.0007	0.0259	0.1128	0.1371	0.0640	0.0854
	$P = .0685$	$P = .6734$	$P = .9944$	$P = .8040$	$P = .2790$	$P = .1877$	$P = .5399$	$P = .4132$
ANA titer	0.1738	0.1209	0.1272	−0.0204	−0.0422	−0.0231	0.0848	−0.0048
	$P = .0720$	$P = .2126$	$P = .1897$	$P = .8339$	$P = .6647$	$P = .8127$	$P = .3827$	$P = .9610$

NOTE. Values with asterisks represent coefficients regarded as significantly correlated when $P < .05$ (lower lane). The value in the upper lane is the correlation coefficient.

[a] AMA titer was available in 94 patients (M2 only in 16 cases).

the new staging system did not show such findings during follow-up. However, no such tendencies were observed in the 2 classical staging systems.

Five of 9 patients of stage 4 in the new system were not cirrhotic (as assessed by histology) at the time of liver biopsy. Two of these 5 cases already presented with any cirrhosis-related condition at the time of liver biopsy, and the other 2 cases developed such conditions during follow-up. The remaining patient was lost to follow-up.

The development of cirrhosis-related conditions and CA and HA grades did not show a significant correlation (data not shown). With respect to the histologic findings that defined the new staging system, a significant correlation was observed between these findings and fibrosis and deposition of orcein-positive granules, but such analyses were not possible because few cases exhibited score 2 or 3 of bile duct loss (Fig. 4A and B). In particular, the rate of development of cirrhosis-related conditions in patients with scores 2 to 3 of deposition of orcein-positive granules was significantly higher than that in patients with scores 0 to 1 ($P < .0001$; Fig. 4C).

4. Discussion

Recently, we proposed a new histologic evaluation system for liver biopsies of PBC to avoid sampling errors and to evaluate necroinflammatory activities. In the present study, we applied this new system to the liver biopsies of 152 patients with PBC. We found different distributions of cases among the 3 systems. In particular, a considerable number of stage 1 cases of the Scheuer/Ludwig systems were reclassified as stage 2 or 3 in the new system because of bile duct loss and/or deposition of orcein-positive granules in the absence of ductular proliferation or interface hepatitis. Histologic heterogeneity in the liver may be a reason for such differences. In addition, several stage 3 cases of the Scheuer/Ludwig systems were reclassified as stage 4 because of extensive bile duct loss or deposition of orcein-positive granules despite the absence of cirrhotic changes. This finding may reflect the more accurate evaluation of pathological progression by our new system (see below).

We then analyzed patients with PBC before UDCA therapy with respect to clinicolaboratory and histologic findings using the 3 staging systems. In this evaluation, the patients who had been used as cases for our previous study [6], in which the new grading and staging system had been proposed, were excluded. The new system and the 2 classical systems were found to correlate well with Alp, γ-GTP, AST, and ALT levels. This system and the Scheuer system also correlated with the total bilirubin level, whereas the Ludwig staging system did not. Instead, the Ludwig staging system correlated with IgG and IgM levels. CA and HA grades also correlated with Alp, γ-GTP, AST, and ALT levels, and interestingly, HA grade correlated with the IgG level, similar to the Ludwig staging system. Taken together, this new staging system combined with HA grade seems

Table 5 Patients with cirrhosis-related conditions

Case no.	"Cirrhosis-related conditions"						Development of cirrhosis-related conditions
	Histologic cirrhosis	Varices	Ascites	HE	HCC	Hyperbilirubinemia	
1	+	ND	+	ND	ND	+	[a]
2	+	ND	ND	ND	ND	ND	[a]
3	ND	ND	ND	ND	+	+	[a]
4	ND	ND	ND	ND	ND	+	[a]
5	ND	+	ND	ND	ND	ND	[a]
6	+	ND	+	ND	ND	ND	[a]
7	ND	ND	+	ND	ND	ND	[a]
8	+	ND	ND	ND	ND	ND	[a]
9	ND	ND	+	ND	ND	+	[a]
10	ND	+	ND	ND	ND	ND	3 [b]
11	ND	+	ND	ND	ND	ND	11 [b]
12	ND	+	ND	ND	ND	ND	21 [b]
13	ND	ND	+	ND	ND	ND	48 [b]
14	ND	+	ND	ND	ND	+	54 [b]
15	ND	ND	+	ND	ND	ND	57 [b]
16	ND	+	ND	ND	ND	ND	60 [b]
17	ND	ND	ND	ND	ND	+	67 [b]
18	+	ND	ND	ND	ND	ND	80 [b]
19	ND	ND	ND	+	ND	ND	107 [b]
20	ND	ND	+	ND	ND	+	122 [b]
21	ND	ND	+	ND	ND	+	182 [b]
22	+	ND	ND	ND	ND	ND	198 [b]
23	ND	ND	+	ND	ND	ND	215 [b]

Abbreviations: HE, hepatic encephalopathy; HCC, hepatocellular carcinoma; +, present; ND, not detected.
[a] Present already at liver biopsy.
[b] Developed after liver biopsy (months).

to reflect liver dysfunction such as cholestasis and the necroinflammatory activities of PBC. However, AMA titer and CA degree, which are both characteristic of PBC, were not associated with each other, and the former was not associated with any of pathological markers examined. This finding supported the notion that AMA was not directly associated with PBC pathogenesis [13].

Then, we evaluated the relationship between this system and prognosis using a retrospective cohort study. With respect to the development of cirrhosis-related conditions, each of these 3 systems, as a whole, showed significance. Interestingly, the development rates increased according to the stage progression of the new system, and significant differences were observed between stages 2 and 3. However, no such tendencies were observed in the classical staging systems. Taken together, this new system was better for predicting outcome (particularly, the development of cirrhosis-related conditions) with respect to prognosis than the other 2 classical staging systems.

With regard to the grading of PBC, such as CA and HA, and the 3 stage-defining findings, the development of cirrhosis-related findings increased along with fibrosis and deposition of orcein-positive granules. Fibrosis, interface hepatitis, and ductopenia/bile duct loss have been reported to be predictors of PBC progression [14-18]. However, the present study results indicated that fibrosis and deposition of orcein-positive granules should be regarded as such predictors, possibly because of the elimination of cases with advanced-stage PBC at the beginning of follow-up in this study. Nonresponders for UDCA have been reported to have a poorer prognosis than do responders [15-17,19,20]. Further studies investigating the relationship of this new system as well as the clinical and biochemical response to UDCA therapy using more patients with PBC seem essential to solve this issue.

Roll et al [18] also reported cholestasis to be an adverse prognostic factor of PBC. Deposition of orcein-positive granules representing copper-binding protein is a very sensitive finding reflecting chronic cholestasis [21]. The present study demonstrated that the amount of deposition of orcein-positive granules was associated with the development of cirrhosis-related conditions. The amount of deposition of orcein-positive granules correlated well with blood biochemical data (Table 4). The score of deposition of orcein-positive granules is necessary to precisely evaluate PBC progression.

In conclusion, we assessed a new staging and grading system for PBC using 152 cases. Some of the cases belonging to stage 1 of the Scheuer/Ludwig system and those to stage 3 of the Scheuer/Ludwig system were reclassified as

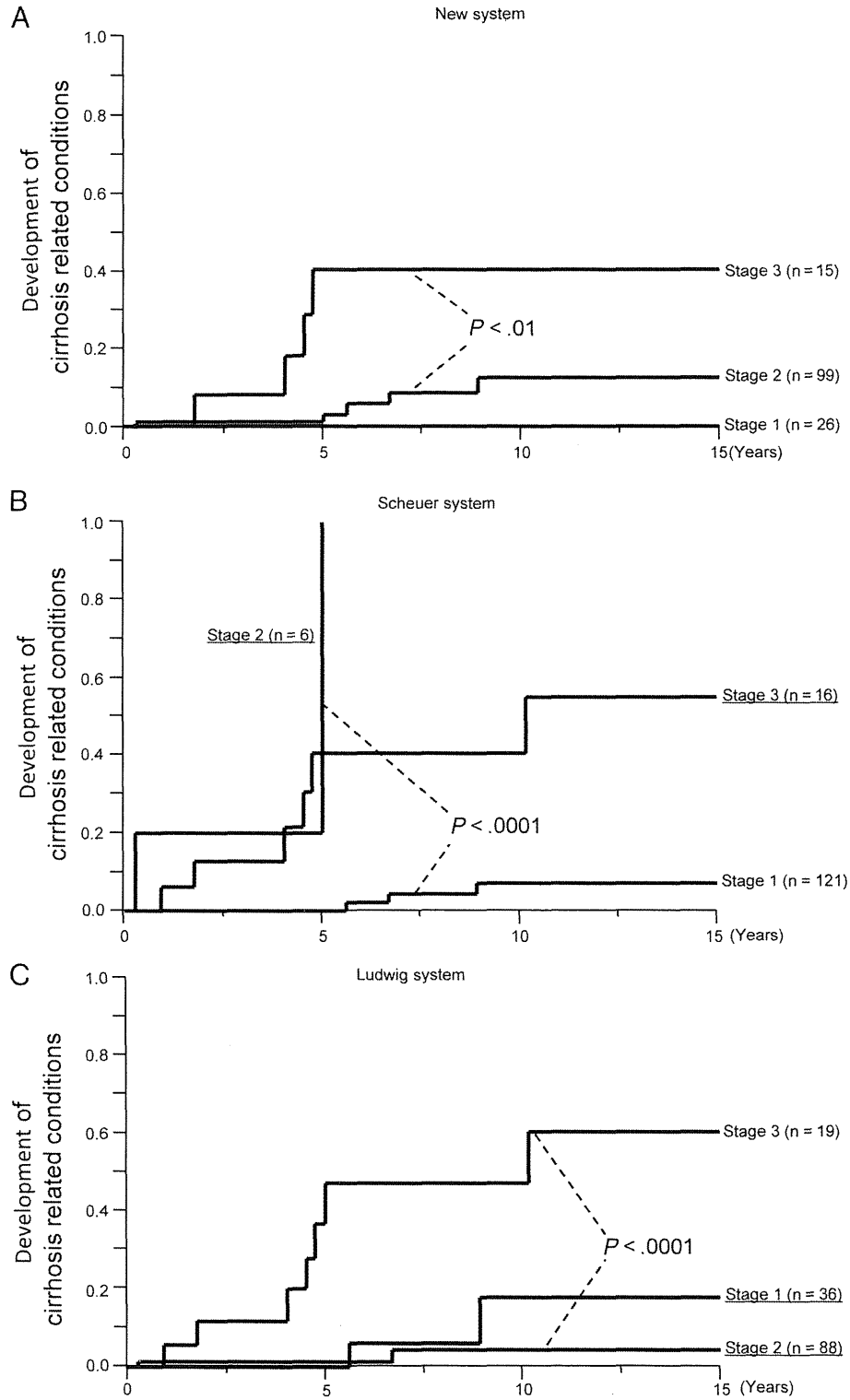

Fig. 3 Rates of development of cirrhosis-related conditions in each histologic stage. A, New staging system. B, Scheuer system. C, Ludwig system. All *P* values were calculated using the log-rank test.

stage 2 or 3 and stage 4 in the new staging, respectively, raising the possibility that an accurate evaluation of pathological progression could be performed using the new system. The new system as well as the 2 classical systems reflected liver dysfunctions before UDCA treatment. The development of cirrhosis-related conditions increased according to the stage progression of the new system on a stepwise basis. Interestingly, deposition of orcein-positive