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著者	Kakuda Yuko, Harada Kenichi, Sawada-Kitamura Seiko, Ikeda Hiroko, Sato Yasunori, Sasaki Motoko, Okafuji Hirofumi, Mizukoshi Eishiro, Terasaki Shuichi, Ohta Hajime, Kasashima Satomi, Kawashima Atsuhiro, Kaizaki Yasuharu, Kaneko Shuichi, Nakanuma Yasuni
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- Title page -

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

Yuko Kakuda,[1] Kenichi Harada,[1] Seiko Sawada-Kitamura,[2] Hiroko Ikeda,[2] Yasunori Sato,[1] Motoko Sasaki,[1] Hirofumi Okafuji,[3] Eishiro Mizukoshi,[3] Shuichi Terasaki,[4] Hajime Ohta,[5] Satomi Kasashima,[6] Atsuhiro Kawashima,[6] Yasuharu Kaizaki,[7] Shuichi Kaneko,[3] and Yasuni Nakanuma[1, 2]

Departments of Human Pathology[1] and Gastroenterology[3], Kanazawa University Graduate School of Medicine, Kanazawa, Japan; Division of Pathology, Kanazawa University Hospital, Kanazawa, Japan[2]; Department of Gastroenterology, Kanazawa Red Cross Hospital, Kanazawa, Japan[4]; Departments of Gastroenterology[5] and Pathology,[6] National Hospital Organization, Kanazawa Medical Center, Kanazawa, Japan; Department of Pathology, Fukui Prefectural Hospital, Fukui, Japan[7]

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Address correspondence to:

Yasuni Nakanuma, MD, PhD

Department of Human Pathology

Kanazawa University Graduate School of Medicine

13-1 Takara-machi, Kanazawa 920-8640, Japan

Tel.: +81-76-265-2195

Fax: +81-76-234-4229

E-mail: nakanuma@staff.kanazawa-u.ac.jp

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Conflict of interest

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List of abbreviations: PBC, primary biliary cirrhosis; CA, cholangitis activity; HA, hepatitis activity; CNSDC, chronic non-suppurative destructive cholangitis; UDCA, ursodeoxycholic acid; Alp, alkaline phosphatase; γ-GTP, gamma-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

Abstract

Recently, our research team proposed a new histological staging and grading system for primary biliary cirrhosis (PBC) that takes into account necroinflammatory activity and histological heterogeneity. The present study aimed to confirm the usefulness of the new evaluating 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 histological components (fibrosis, bile duct loss, and deposition of orcein-positive granules), and grading was assessed on the basis of chronic cholangitis activity (CA) and hepatitis activity (HA). Concurrently, the classical systems, i.e., 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 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 vs. scores 2–3 groups $p < 0.0001$). 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 non-suppurative destructive cholangitis (CNSDC). After cholangiopathy, the affected bile ducts finally disappear, causing cholestatic liver failure and cirrhosis (1, 2).

Several histological 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 histological feature (e.g., 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 histological 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 pre-cirrhotic stages are not.

Recently, we proposed a new grading and staging system for PBC that takes into account the histological 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 with 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), gamma-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, i.e., a portal tract having a portal vain 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 histological staging and grading system for PBC

Representative histological findings of PBC as defined by our new histological 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 minimum progression), 1–3 was stage 2 (mild progression), 4–6 was stage 3 (moderate progression), and 7–9 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.

Cholangitis activity (CA): 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-oriented lymphoplasmacytic infiltration and/or periductal epithelioid granulomas (Fig. 1A and 1B). In contrast, “evident chronic cholangitis” was defined as non-specific 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 one damaged bile duct showing CNSDC or a florid duct lesion (3, 4) was found.

Hepatitis activity (HA): "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 HA grading.

2.3. Histological 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) (i.e., stage 1: florid duct lesions or CNSDC; stage 2: proliferation of bile ductules; stage 3: fibrosis or scarring; and stage 4: cirrhosis) and the Ludwig system (5) (i.e., 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 1/5 of cases among the 3 pathologists, but after discussion, a consensus regarding the appropriate grade was reached.

2.4. Definitions of endpoint

The terminal morphological 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 endpoint 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, i.e., 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 ± standard deviation. Correlations and comparisons of biochemical data of each grade, score, or stage were examined using Spearman's 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 $p < 0.05$ was considered significant. All statistical analyses were performed using JMP software 8.0 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Distribution of histological 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 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–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 PBCsuch 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 two staging systems are shown in Fig. 2F–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 histological lesions (i.e., fibrosis, bile duct loss, or deposition of orcein-positive 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's 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, AMA and antinuclear antibody titers did not show significant correlation with any staging systems, HA and CA grades, or other histological lesions for staging.

3.3. Histological 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 the hospital immediately after liver biopsy and could not be followed up. A total of 23 patients presented with one or several cirrhosis-related conditions (Table 5), and such condition(s) 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 stage 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 ($p < 0.01$). 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 ($p < 0.01$). Moreover, stage 1 patients of 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 histological 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 2–3 of deposition of orcein-positive granules was significantly higher than that in patients with scores 0–1 ($p < 0.0001$) (Fig. 4C).

4. Discussion

Recently, we proposed a new histological 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. Histological 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 PBC patients before UDCA therapy with respect to clinicolaboratory and histological 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 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 predictor, possibly because of the elimination of cases with advanced-stage PBC at the beginning of follow-up in this study. Non-responders for UDCA have been reported to have a poorer prognosis than 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 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 reflected liver dysfunctions before UDCA treatment as well as the 2 classical systems. The development of cirrhosis-related conditions increased according to the stage progression of the new system on a step-wise basis. Interestingly, deposition of orcein-positive granules was a useful predictive factor for the development of cirrhosis-related conditions and was eventually a useful prognostic factor for patients with PBC. Studies with larger cohorts involving different institutions seem to be necessary to more accurately validate this new histological grading and staging system.

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Figure legends

Fig. 1 Histological findings defining the staging and grading of PBC. (A) Chronic non-suppurative destructive cholangitis 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) A, hepatic artery, P, portal vein (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 <1/3 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 >2/3 of portal tracts in the liver biopsy specimen (score 3) P, portal vein. (orcein stain, 400× magnification). Score 2 of deposition of orcein-positive granules represents the amount of deposition in zone 1 hepatocytes in 1/3 to 2/3 of portal tracts between (F) (score 1) and (G) (score 3).

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

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

Fig. 4 Rates of development of cirrhosis-related conditions in each score of (A) fibrosis and (B and C) deposition of orcein-positive granules. (C) is the comparison with scores 0–1 vs. scores 2–3 of deposition of orcein-positive granules. All *p* values were calculated using the log rank test.

Table 1. Scoring for the staging of primary biliary cirrhosis

A. Fibrosis

Score	Criterion
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

Score	Criterion
0	No bile duct loss
1	Bile duct loss in <1/3 of portal tracts
2	Bile duct loss in 1/3–2/3 of portal tracts
3	Bile duct loss in >2/3 of portal tracts

C. Deposition of orcein-positive granules [a]

Score	Criterion
0	No deposition of granules
1	Deposition of granules in a couple of zone 1 hepatocytes at <1/3 of portal tracts
2	Deposition of granules in a variable number of zone 1 hepatocytes at 1/3–2/3 of portal tracts
3	Deposition of granules in most zone 1 hepatocytes at >2/3 of portal tracts

a) See Fig. 1F and G.

Table 2. Grading of the necroinflammatory activity of primary biliary cirrhosis

A. Cholangitis activity (CA)

Grade	Criteria
0 (no activity)	No cholangitis but mild damage to the epithelium of the duct may be present
1 (mild activity)	One evident chronic cholangitis in the specimen
2 (moderate activity)	More than two bile ducts with evident chronic cholangitis
3 (marked activity)	At least one CNSDC in the specimen

B. Hepatitis activity (HA)

Grade	Criteria
0 (no activity)	No interface hepatitis and no or minimum lobular hepatitis
1 (mild activity)	Interface hepatitis affecting 10 continuous hepatocytes at a limiting plate in one portal tract or fibrous septa and mild-to-moderate lobular hepatitis
2 (moderate activity)	Interface hepatitis affecting 10 continuous hepatocytes at a limiting plates in more than two portal tracts or fibrous septa and mild-to-moderate lobular hepatitis
3 (marked activity)	Interface hepatitis affecting 20 continuous hepatocytes at limiting plate in more than half of the portal tracts and modetate lobular hepatitis or bridging/zonal necrosis

Abbreviation: CNSDC, chronic non-suppurative destructive cholangitis

Table 3. Clinical characteristics of patients

	Total (n = 152)	(n = 110) [a]
Age (mean ± SD, years)	57 ± 12	56 ± 12
Sex (males : females)	17:135	11:99
Observation period (mean ± SD, years)	5.6 ± 5.8 (range, 0–24.8 years)	5.7 ± 6.0 (range, 0–24.8 years)
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 (mean ± SD, IU/L)	592 ± 501	522 ± 374
γ-GTP (mean ± SD, IU/L)	224 ± 203	221 ±207
AST (mean ± SD, IU/L)	49.7 ± 31.5	47.6 ± 28.4
ALT (mean ± SD, IU/L)	50.6 ± 38.6	50.7 ± 38.3
Total bilirubin (mean ± SD, mg/dL)	0.89 ± 1.21	0.76 ± 0.43
Albumin (mean ± SD, g/dL)	4.16 ± 0.56 [d]	4.21 ± 0.48
Prothrombin time (mean ± SD, sec)	11.4 ± 0.81 [d]	11.6 ± 0.61
IgG (mean ± SD, mg/dL)	1897 ± 611	1798 ± 536
IgM (mean ± SD, mg/dL)	376 ± 267	370 ± 280

[a] Cases which had not been used in our previous study (6). [b] ANA was available for 151 patients. [c] 13 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). Abbreviations: SD, standard deviation; AMA, antimitochondrial antibodies; M2Ab, M2 antibodies; ANA, antinuclear antibodies

Table 4. Analysis between grading and staging systems and laboratory data for primary biliary cirrhosis using Spearman's 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 = 0.0062*	p = 0.0336*	p = 0.0082*	p = 0.0006*	p < 0.0001*	p < 0.0001*	p < 0.0001*	p = 0.0286*
γ-GTP	0.2061*	0.2004*	0.3923*	0.3029*	0.4265*	0.4322*	0.4204*	0.2272*
	p = 0.0323*	p = 0.0376*	p < 0.0001*	p = 0.0014*	p < 0.0001*	p < 0.0001*	p < 0.0001*	p = 0.0181*
AST	0.2722*	0.3915*	0.3884*	0.2264*	0.3766*	0.4209*	0.4476*	0.4019*
	p = 0.0044*	p < 0.0001*	p < 0.0001*	p = 0.0185*	p < 0.0001*	p < 0.0001*	p < 0.0001*	p < 0.0001*
ALT	0.2580*	0.3797*	0.3289*	0.1754	0.3238*	0.4363*	0.4139*	0.3804*
	p = 0.0070*	p < 0.0001*	p = 0.0005*	p = 0.0694	p = 0.0006*	p < 0.0001*	p < 0.0001*	p < 0.0001*
Total bilirubin	−0.0192	0.0119	0.0815	0.1626	0.2687*	0.2034*	0.2695*	0.0937
	p = 0.8443	p = 0.9028	p = 0.4041	p = 0.0943	p = 0.0051*	p = 0.0356*	p = 0.0050*	p = 0.3371
IgM	0.1156	−0.0104	0.1226	0.1270	0.1590	0.1793	0.1949*	0.1909*
	p = 0.2334	p = 0.9146	p = 0.2062	p = 0.1901	p = 0.1003	p = 0.0634	p = 0.0432*	p = 0.0478*
IgG	−0.0241	0.2122*	0.0889	0.1868	0.0845	0.0782	0.0690	0.2105*
	p = 0.8070	p = 0.0297*	p = 0.3674	p = 0.0563	p = 0.3916	p = 0.4278	p = 0.4843	p = 0.0311*
AMA titer [a]	−0.1887	0.0440	−0.0007	0.0259	0.1128	0.1371	0.0640	0.0854
	p = 0.0685	p = 0.6734	p = 0.9944	p = 0.8040	p = 0.2790	p = 0.1877	p = 0.5399	p = 0.4132
ANA titer	0.1738	0.1209	0.1272	−0.0204	−0.0422	−0.0231	0.0848	−0.0048
	p = 0.0720	p = 0.2126	p = 0.1897	p = 0.8339	p = 0.6647	p = 0.8127	p = 0.3827	p = 0.9610

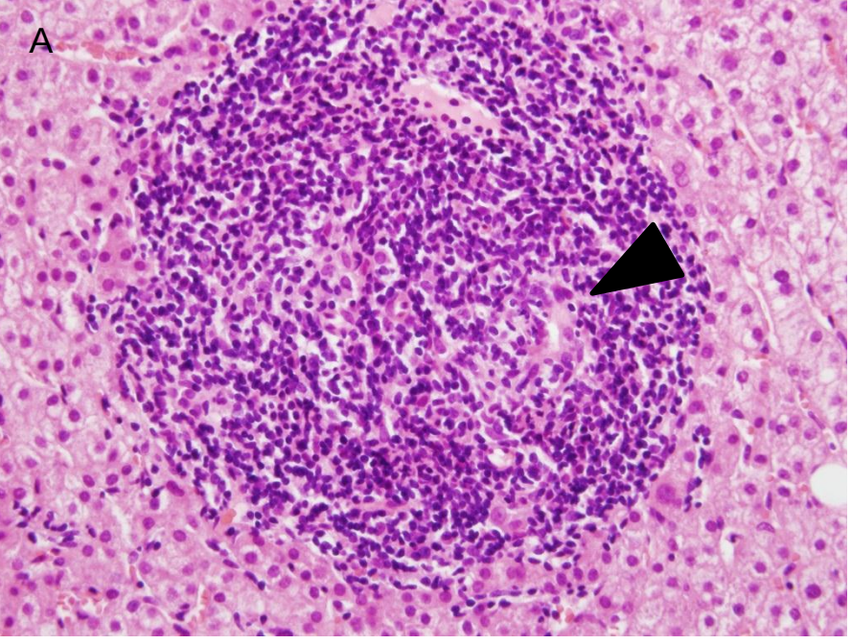
Values with asterisks represent coefficients regarded as significantly correlated when $p < 0.05$ (lower lane). The value in the upper lane is the correlation coefficient. [a]AMA titer was available in 94 patients (M2 only in 16 cases).

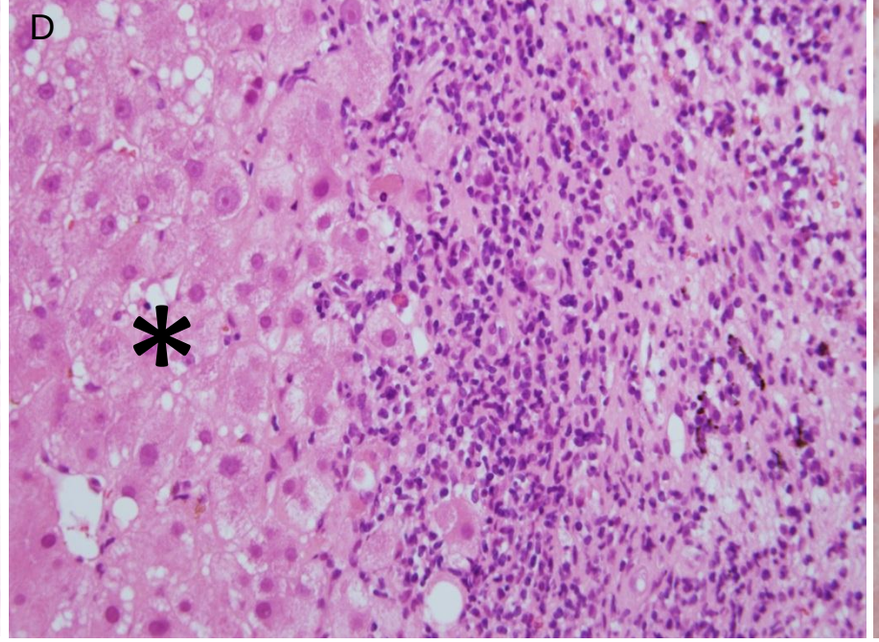
Table 5. Patients with cirrhosis-related conditions

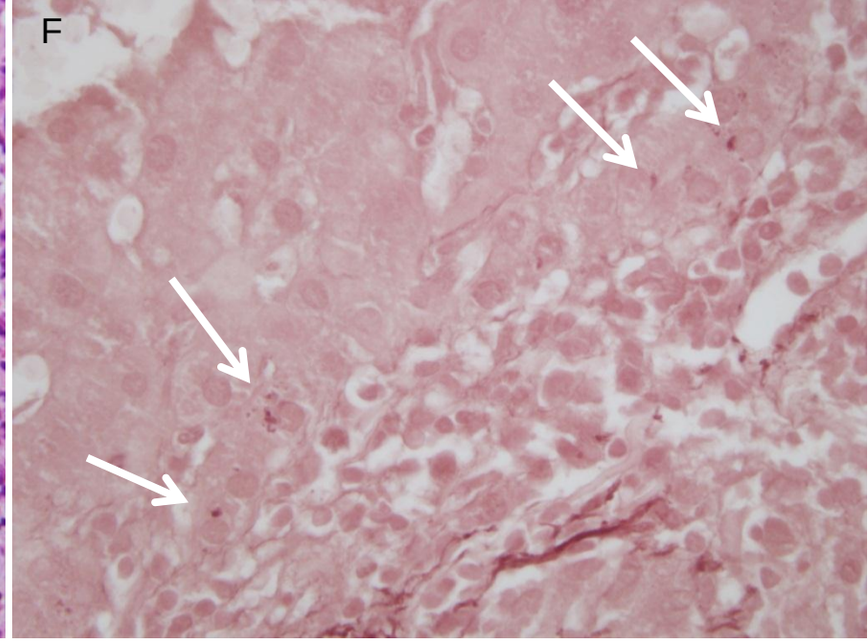
Case No.	"Cirrhosis-related conditions"						Development of "cirrhosis-related conditions"
	Histological cirrhosis	Varices	Ascites	HE	HCC	Hyper-bilirubinemia	
1	+	n.d.	+	n.d.	n.d.	+	*
2	+	n.d.	n.d.	n.d.	n.d.	n.d.	*
3	n.d.	n.d.	n.d.	n.d.	+	+	*
4	n.d.	n.d.	n.d.	n.d.	n.d.	+	*
5	n.d.	+	n.d.	n.d.	n.d.	n.d.	*
6	+	n.d.	+	n.d.	n.d.	n.d.	*
7	n.d.	n.d.	+	n.d.	n.d.	n.d.	*
8	+	n.d.	n.d.	n.d.	n.d.	n.d.	*
9	n.d.	n.d.	+	n.d.	n.d.	+	*
10	n.d.	+	n.d.	n.d.	n.d.	n.d.	3**
11	n.d.	+	n.d.	n.d.	n.d.	n.d.	11**
12	n.d.	+	n.d.	n.d.	n.d.	n.d.	21**
13	n.d.	n.d.	+	n.d.	n.d.	n.d.	48**
14	n.d.	+	n.d.	n.d.	n.d.	+	54**
15	n.d.	n.d.	+	n.d.	n.d.	n.d.	57**
16	n.d.	+	n.d.	n.d.	n.d.	n.d.	60**
17	n.d.	n.d.	n.d.	n.d.	n.d.	+	67**
18	+	n.d.	n.d.	n.d.	n.d.	n.d.	80**
19	n.d.	n.d.	n.d.	+	n.d.	n.d.	107**
20	n.d.	n.d.	+	n.d.	n.d.	+	122**
21	n.d.	n.d.	+	n.d.	n.d.	+	182**
22	+	n.d.	n.d.	n.d.	n.d.	n.d.	198**
23	n.d.	n.d.	+	n.d.	n.d.	n.d.	215**

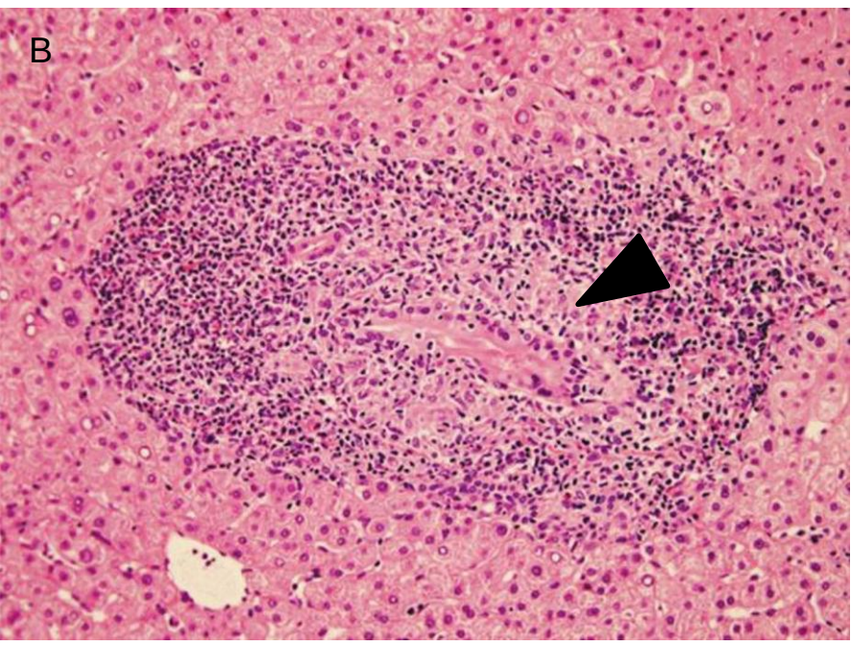
Abbreviations: HE, Hepatic encephalopathy; HCC, Hepatocellular carcinoma; +, present; n.d., not detected.

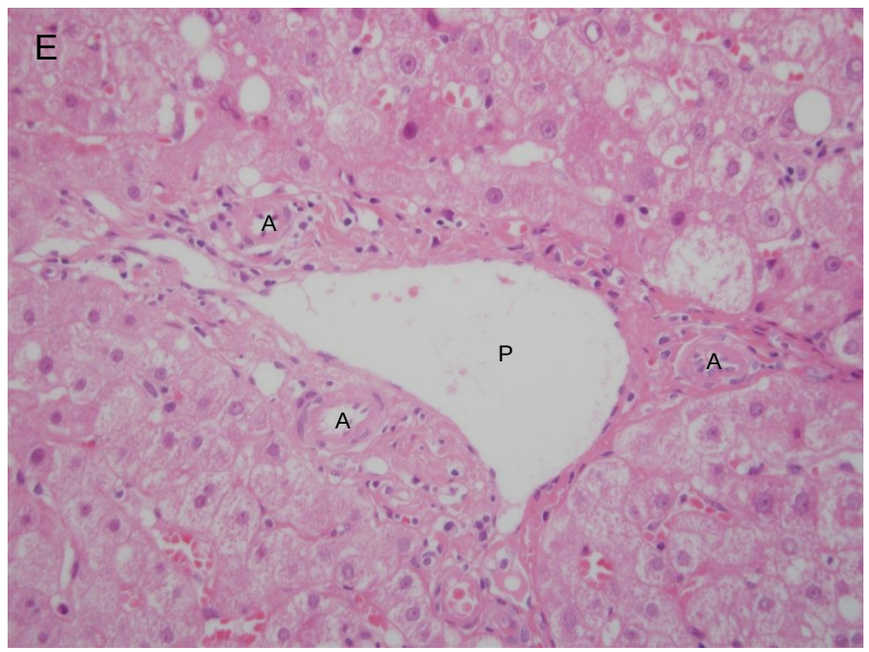
*, present already at liver biopsy; **, developed after liver biopsy (months)

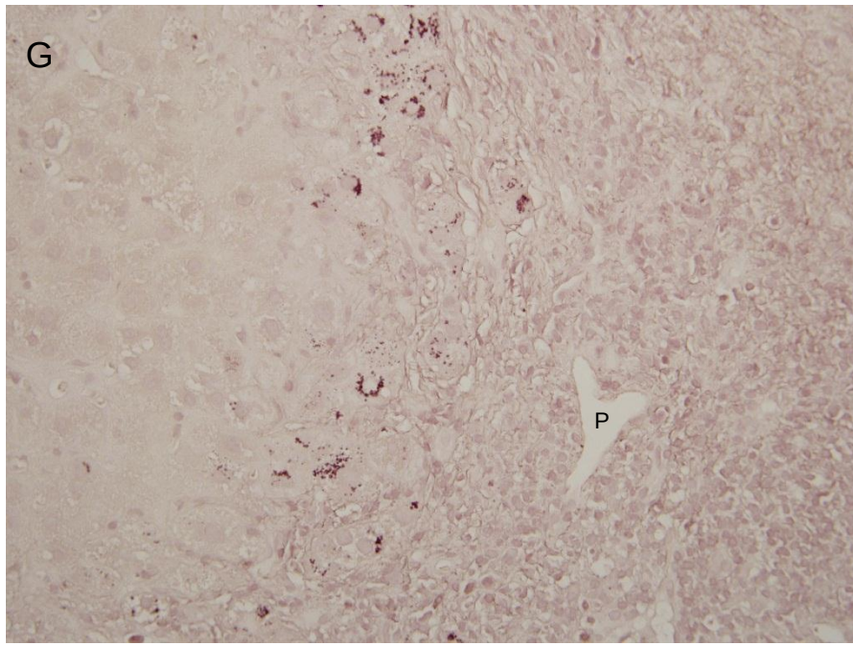












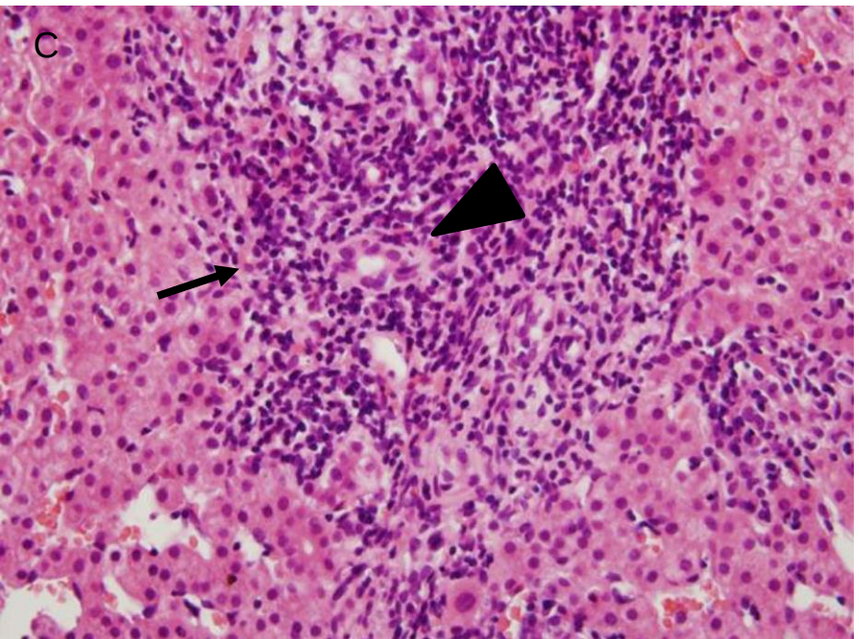


Fig. 1

Fig.2

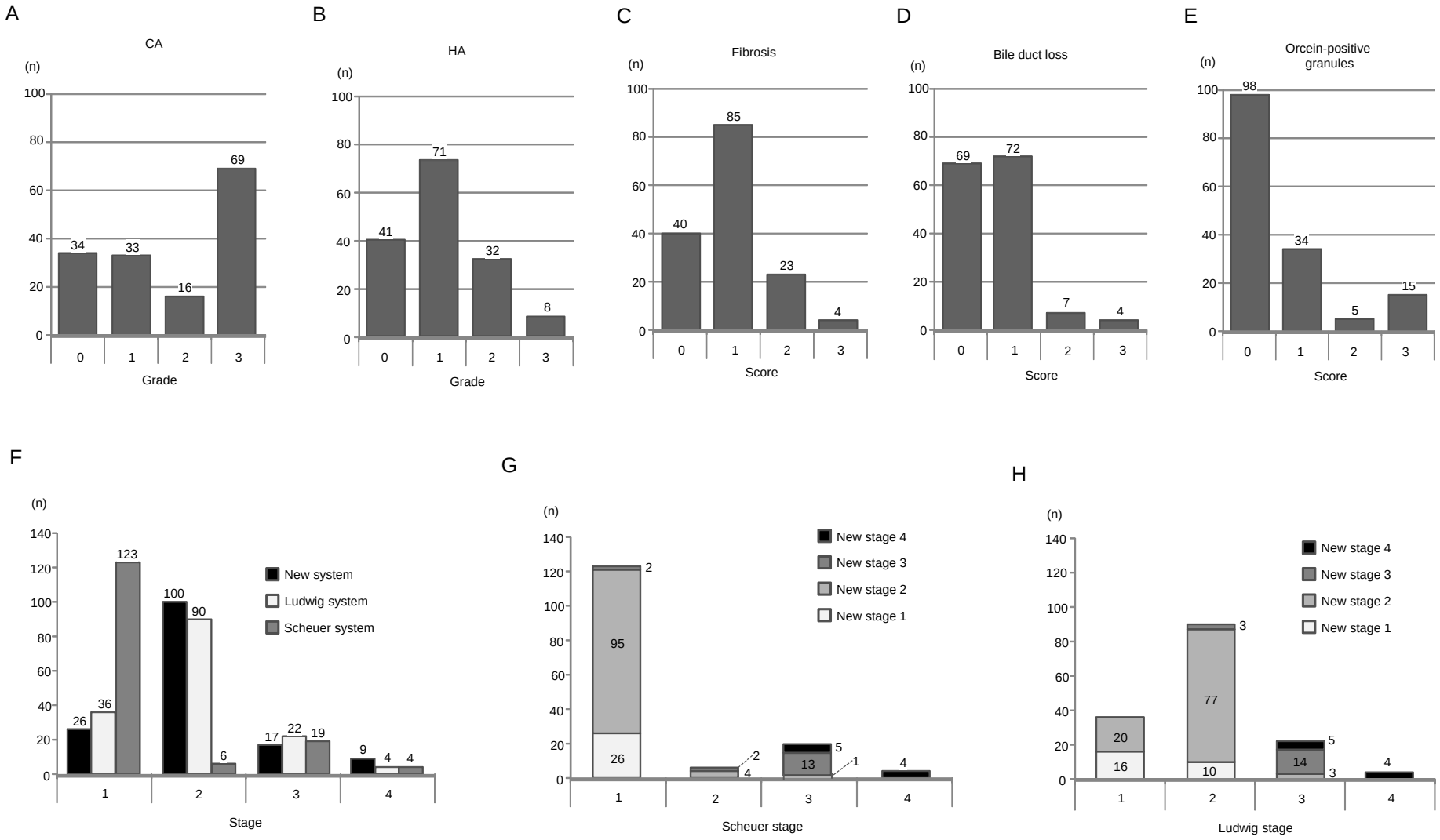
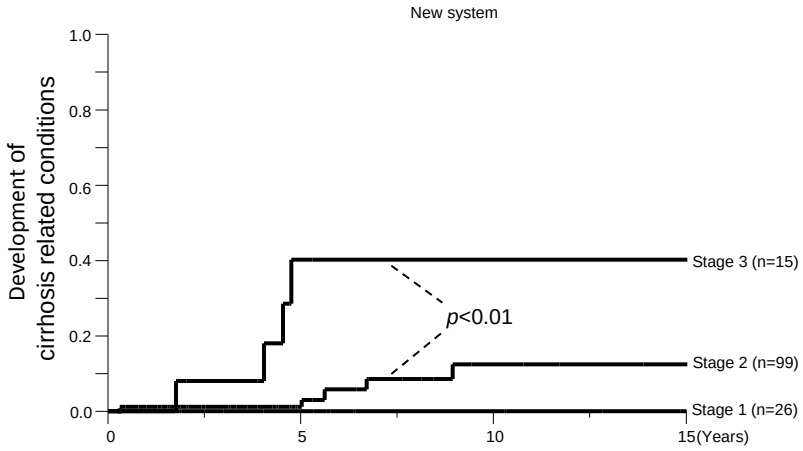
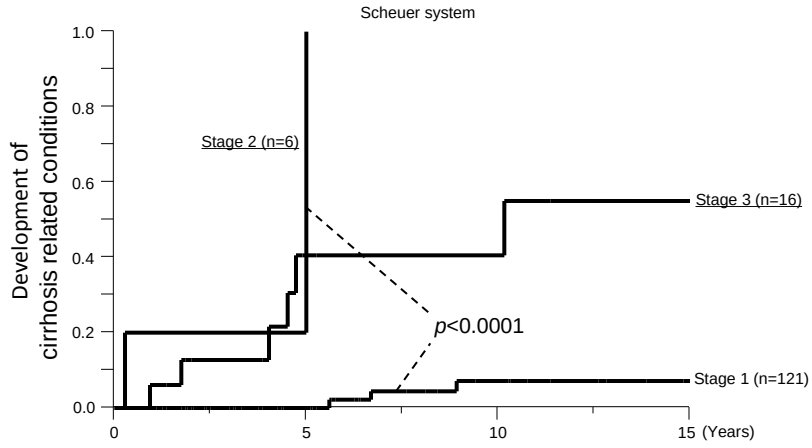

A
CA
(n)
100
80
60
40
20
0
34
33
16
69
0
1
2
3
Grade
B
HA
(n)
41
71
32
8
Grade
C
Fibrosis
(n)
40
85
23
4
Score
D
Bile duct loss
(n)
69
72
7
4
Score
E
Orcein-positive granules
(n)
98
34
5
15
Score
F
(n)
140
120
26
36
123
100
90
6
17
22
19
9
4
4
New system
Ludwig system
Scheuer system
1
2
3
4
Stage
G
(n)
95
26
2
4
2
5
13
1
1
4
New stage 4
New stage 3
New stage 2
New stage 1
Scheuer stage
H
(n)
20
16
77
10
3
14
5
3
4
New stage 4
New stage 3
New stage 2
New stage 1
Ludwig stage

Fig. 3

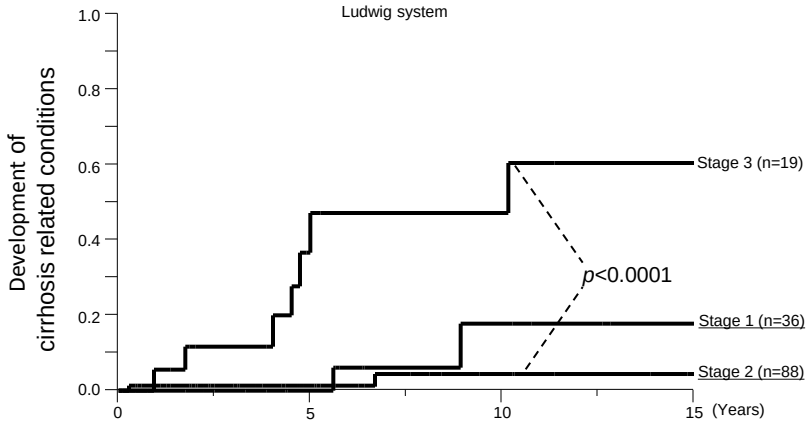
A

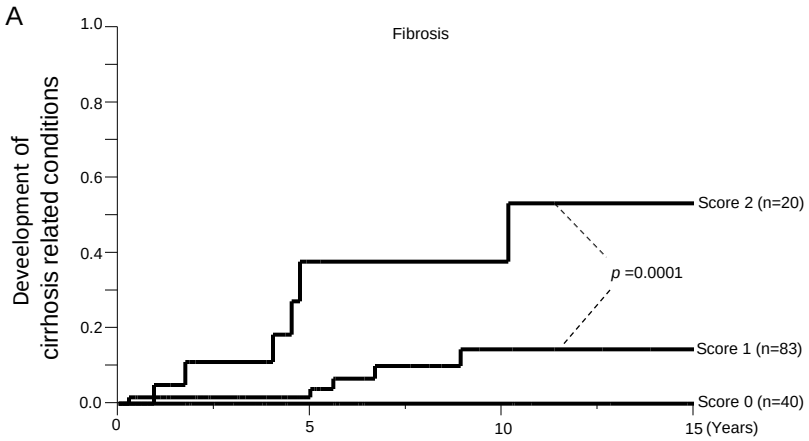

New system
Development of cirrhosis related conditions
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15(Years)
Stage 3 (n=15)
p<0.01
Stage 2 (n=99)
Stage 1 (n=26)

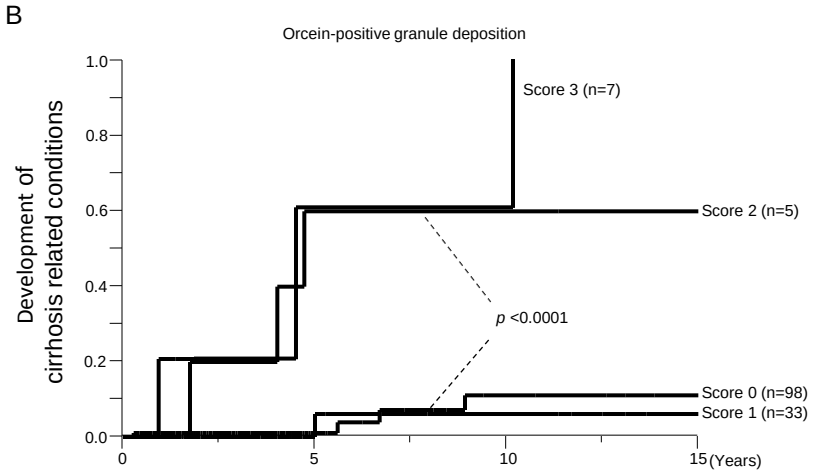
B

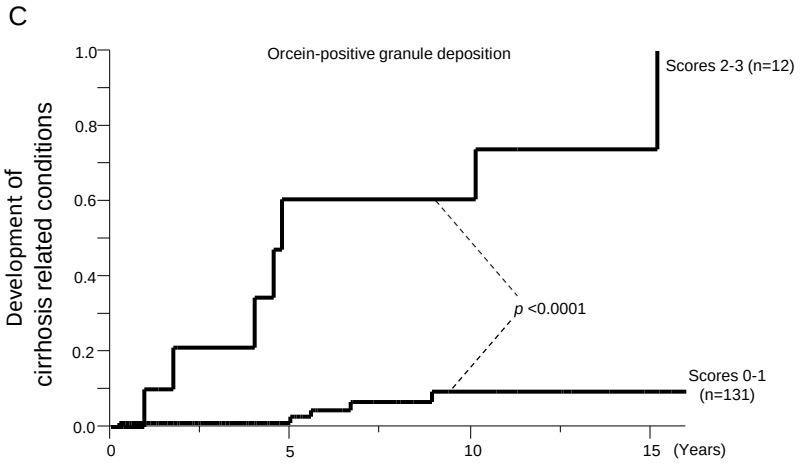

Scheuer system
Development of cirrhosis related conditions
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15 (Years)
Stage 2 (n=6)
Stage 3 (n=16)
p<0.0001
Stage 1 (n=121)

C


Ludwig system
Development of cirrhosis related conditions
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15 (Years)
Stage 3 (n=19)
p<0.0001
Stage 1 (n=36)
Stage 2 (n=88)


A
Fibrosis
Deveelopment of cirrhosis related conditions
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15 (Years)
Score 2 (n=20)
p =0.0001
Score 1 (n=83)
Score 0 (n=40)


B
Orcein-positive granule deposition
Development of cirrhosis related conditions
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15 (Years)
Score 3 (n=7)
Score 2 (n=5)
p <0.0001
Score 0 (n=98)
Score 1 (n=33)


C
Orcein-positive granule deposition
Development of cirrhosis related conditions
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15 (Years)
Scores 2-3 (n=12)
p <0.0001
Scores 0-1 (n=131)