

Article

## Impact of Aging on Liver Histological Findings of Autoimmune Liver Diseases

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**Abstract:** Our aim is to investigate the recent liver biopsy findings of autoimmune liver diseases at a university hospital located in an urban area of Japan. The study included 259 patients (mean age  $56.8 \pm 12.5$ ; male/female, 46/213) who underwent a liver biopsy for primary biliary cirrhosis (PBC) or autoimmune hepatitis (AIH). We analyzed their liver biopsy findings according to age and gender. Among 127 PBC patients, Scheuer stages 1, 2, 3, and 4 were 42, 54, 18, and 13, respectively. Among 101 AIH patients, fibrosis stages F1, F2, F3, and F4 were 37, 32, 19, and 13, respectively, and inflammatory activity grades A1, A2, and A3 were 22, 25, and 54, respectively. Among PBC aged  $\geq 65$  years, Scheuer stages 1–3 and 4 patients were 27 and 6, respectively. The proportion of Scheuer stage 4 patients in PBC aged  $\geq 65$  years tended to be higher than that in PBC aged  $< 65$  years ( $p = 0.0659$ ). Of interest, the proportion of AIH patients with moderate or severe activity (A2 or A3) in males was higher than in females ( $p = 0.0311$ ). From the point of view of fibrosis stage or inflammatory activity grade of the liver, the proportion of AIH patients aged  $\geq 65$  years was similar to that aged  $< 65$  years. Although we identified six older cirrhotic patients with AIH, three of them were male. The progression of fibrosis and

inflammatory activity of the liver should be noted when we treat older patients suffering from autoimmune liver diseases. Liver biopsy plays an important role in obtaining accurate information on autoimmune liver diseases in older patients.

**Keywords:** autoimmune hepatitis; hepatic fibrosis; liver biopsy; primary biliary cirrhosis; staging of fibrosis

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## 1. Introduction

Primary biliary cirrhosis (PBC) is a progressive chronic cholestatic liver disease, characterized by non-suppurative cholangitis, that mainly affects middle-aged women [1,2]. The reported number of patients with PBC has increased dramatically in the United States [2], United Kingdom [3], and Japan [4,5]. Understanding of the pathophysiology and earlier diagnosis of PBC has increased its prevalence worldwide [6]. The natural history of PBC has improved greatly during the past two decades because of its diagnosis at earlier stages and the widespread use of ursodeoxycholic acid (UDCA) [7,8]. As a result, far fewer patients require liver transplantation [7].

Autoimmune hepatitis (AIH) is characterized by chronic inflammatory liver disease, which, when not treated with prednisolone alone or in combination with azathioprine, leads to liver cirrhosis. It also affects mainly women [9]. AIH can occasionally have clinical, laboratory, histological, and genetic characteristics identical to those of PBC [10]. Some cases of AIH are difficult to diagnose [10–14]. The incidence of AIH differs in various geographic regions. The incidence of type 1 AIH among Caucasoid populations in Europe and North America ranges from 0.1 to 1.9/100,000/year [15]. A population-based epidemiological study of AIH in New Zealand demonstrated that age-standardized incidence and prevalence were 1.7 and 18.9 per 100,000, respectively [16]. In Japan, the prevalence is as high as 1.0 per 10,000 [17]. Except for atypical presentations of AIH [12,18,19], early administration of corticosteroid usually leads to a relatively better prognosis for this disease.

Histological assessment of the liver is valuable for evaluation and management of patients with liver diseases even if sensitive and relatively accurate blood testing is available [20]. Liver biopsy also plays an important role in the diagnosis, staging, prognosis and management of PBC and AIH [20]. In Japan, recent opportunities to manage patients with autoimmune liver diseases such as AIH and PBC have been increasing. This tendency is especially apparent in the older population, as many more people live to an advanced age in Japan. For older patients with liver diseases, the risk of cirrhosis and hepatocellular carcinoma (HCC) is more critical than for the young [21], although there is a contrary opinion [22]. In the present study, to clarify the features of older patients with autoimmune liver diseases at a university hospital located in urban Japan, we analyzed the histological features of these diseases by reviewing the recent findings of liver biopsies.

## 2. Experimental Section

### 2.1. Research Subjects

Between 2000 and 2011, 259 patients were diagnosed with AIH and PBC through liver biopsy specimens; we reviewed and re-analyzed these liver biopsy findings at the Department of Gastroenterology and Nephrology, Chiba University, Graduate School of Medicine, Japan. All subjects gave their informed consent for inclusion before participating in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Chiba University School of Medicine (No. 1841).

### 2.2. Liver Biopsy

We conducted liver biopsies as follows: a patient lay face-up on a bed, right hand resting above the head. The patient was given a local anesthetic to the body surface area where the biopsy needle would be inserted. The patient was administered 15 mg of pentazocine hydrochloride, 0.25 mg of atropine sulfate, and 25 mg of hydroxyzine pamoate as premedication. We made a small incision in the right side of the patient's abdomen, just below the rib cage. Liver biopsy was performed with 14–20 gauge automatic or manual Tru-cut biopsy needles under ultrasound guidance. Biopsy samples were immediately placed into 10% formalin solution (Wako Pure Chemical, Osaka, Japan).

### 2.3. Evaluation of Biopsy Samples

Three hepatologists blindly evaluated the histopathological findings of liver biopsy samples with hematoxylin and eosin (HE) stain. Scheuer's classification was used for evaluation of histopathological findings of PBC: a lesion characteristic of bile ducts was classified as Stage I; florid bile duct lesions were judged as Stage II; and hepatic fibrosis and cirrhosis were categorized as Stages III and IV, respectively [23]. The liver tissue samples from AIH patients were evaluated with fibrosis staging and inflammatory activity grading of the liver in chronic liver diseases [24]. We classified samples from AIH patients into no fibrosis (F0), mild fibrosis (F1), moderate fibrosis (F2), severe fibrosis (F3), and cirrhosis (F4); or minimal activity (A0), mild activity (A1), moderate activity (A2), and severe activity (A3) for staging the extent of fibrosis or grading the activity of inflammation, respectively.

### 2.4. Statistical Analysis

For all tests, two-sided P-values were calculated and the results were considered statistically significant at  $p < 0.05$ . Statistical analysis was performed using the Excel statistics program for Windows, version 7 (SSRI, Tokyo, Japan) and DA Stats software (O. Nagata, Nifty Serve: PAF01644).

### 3. Results

#### 3.1. Patient Characteristics

A total of 259 patients with autoimmune liver diseases were enrolled into the present study (Table 1). Female patients dominated (82.2%) the patient groups: 81.7% and 82.9% of PBC and AIH patients, respectively. According to our records, age was unknown in nine patients with PBC and 35 patients with AIH, although liver biopsy was still performed on these patients. The number of older patients,  $\geq 65$  years, was at least 27.4%: 25.2% and 29.7% of PBC and AIH patients, respectively. Liver biopsy findings from four PBC and 27 AIH patients were not available.

**Table 1.** Characteristics of patients with autoimmune liver diseases in the present study.

	Total Patients (n = 259)	PBC (n = 131)	AIH (n = 128)	p-values
Age (years)	56.8 $\pm$ 12.5	57.2 $\pm$ 10.7	56.5 $\pm$ 14.2	N.S.
Older Patients ( $\geq 65$ years)	71	33	38	N.S.
Gender (male/female)	46/213	24/107	22/106	N.S.
Histopathological findings				
Staging of Fibrosis (F1/F2/F3/F4)	-	-	37/32/19/13	-
Grading of Activity (A1/A2/A3)	-	-	22/25/54	-
Scheuer Stage (1/2/3/4)	-	42/54/18/13	-	-

Note: PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis.

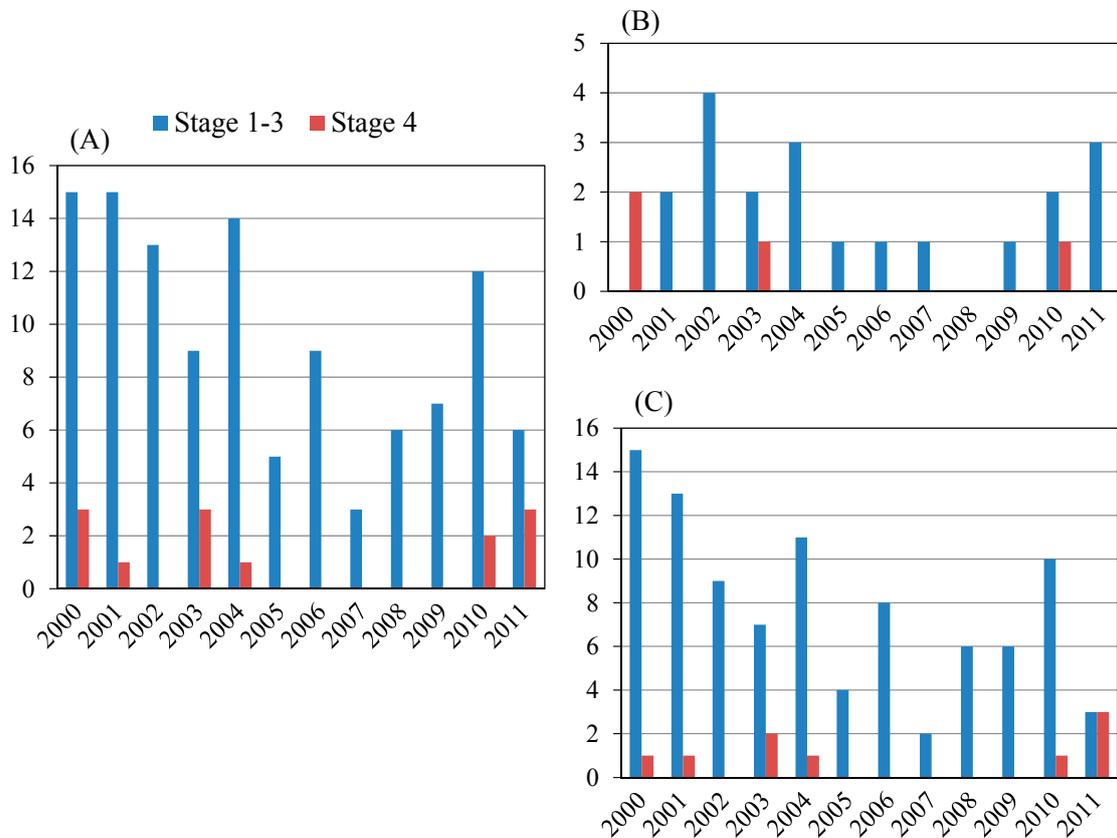
#### 3.2. Annual Changes in the Number of PBC Patients with Liver Biopsy

In the present study, the total number of PBC was 131. Liver biopsy was performed in 127 of 131 patients in the years shown in Figure 1A. Between three and 15 PBC cases, including 13 with Scheuer stage 4, were biopsied in each year. Among them, males and females were 24 and 103, respectively (Figure 1B,C). Among the 13 patients with Scheuer stage 4, four were males and nine were females.

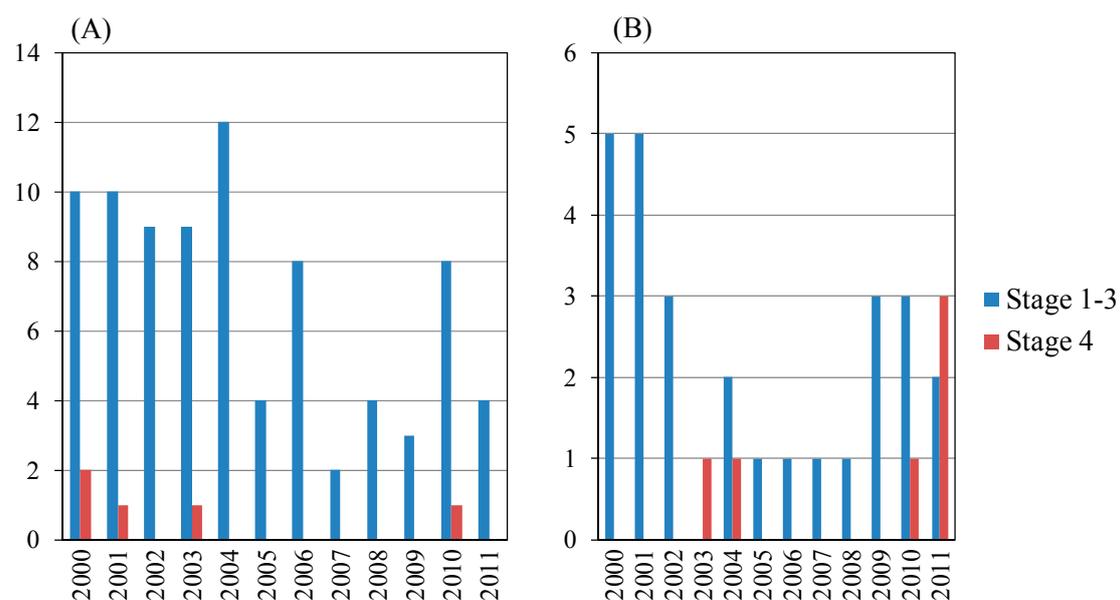
#### 3.3. Older PBC Patients Tended to Have Advanced Fibrosis

PBC patients aged  $< 65$  years and  $\geq 65$  years numbered 91 and 33, respectively (Figure 2A,B). Among PBC patients aged  $< 65$  years, Scheuer stages 1–3 and stage 4 patients numbered 86 and five, respectively. Among PBC patients aged  $\geq 65$  years, Scheuer stages 1–3 and stage 4 patients numbered 27 and 6 respectively. The proportion of Scheuer stage 4 patients aged  $\geq 65$  years tended to be higher than that in those aged  $< 65$  years ( $p = 0.0659$ ). The 11 PBC patients with Scheuer stage 4 are shown in Table 2A. All six patients aged  $\geq 65$  years with Scheuer stage 4 were female.

**Figure 1.** Scheuer stage of primary biliary cirrhosis (PBC) patients in the present study. (A) Total patients ( $n = 127$ ), Stage 1–3: Stage 4 = 114:13; (B) Male patients ( $n = 24$ ), Stage 1–3: Stage 4 = 20:4; (C) Female patients ( $n = 103$ ), Stage 1–3: Stage 4 = 94:9.



**Figure 2.** Scheuer stage of primary biliary cirrhosis (PBC) patients according to age. (A) Age <65 (years) ( $n = 91$ ); (B) Age  $\geq 65$  (years) ( $n = 33$ ).



**Table 2.** Cirrhotic patients of primary biliary cirrhosis and autoimmune hepatitis in the present study. **(A)** Patients with Scheuer stage 4 of primary biliary cirrhosis; **(B)** Cirrhotic patients with autoimmune hepatitis.

<b>(A)</b>		
<b>Year of Diagnosis</b>	<b>Age at Diagnosis (years)</b>	<b>Gender</b>
2000	49	Male
2000	57	Female
2001	42	Female
2003	60	Female
2010	59	Male
2003	80	Female
2004	68	Female
2010	70	Female
2011	73	Female
2011	78	Female
2011	80	Female
<b>(B)</b>		
<b>Year of diagnosis</b>	<b>Age at diagnosis (years)</b>	<b>Gender</b>
2001	58	Female
2002	45	Female
2004	62	Female
2004	61	Female
2006	36	Male
2011	58	Female
2000	67	Male
2004	68	Female
2005	72	Male
2005	74	Male
2009	70	Female
2009	70	Female

#### 3.4. Annual Changes in the Number of AIH Patients with Liver Biopsy

In the present study, the total number of AIH was 128. Table 3A shows the yearly schedule of liver biopsy of 101 of the 128 patients. Among them, males and females numbered 18 and 83, respectively (Table 3A,B). Between 2000 and 2011, the annual number of AIH was between six and 16 cases, including 13 cirrhotic patients (four males and nine females). The proportion of cirrhotic patients with AIH did not differ statistically between males and females (Table 3A). The proportion of AIH patients with moderate or severe activity (A2 or A3) was higher for males than for females ( $p = 0.0311$ ) (Table 3B). Of interest, among the AIH patients, all male patients had A2 or A3 inflammatory activity grade of the liver.

**Table 3.** Histopathological findings of the liver in autoimmune hepatitis according to gender in the present study. **(A)** Staging of fibrosis; **(B)** Grading of inflammatory activity.

<b>(A)</b>						
	<b>Total Patients (n = 101)</b>		<b>Male (n = 18)</b>		<b>Female (n = 83)</b>	
Year	F1-F3	F4	F1-F3	F4	F1-F3	F4
2000	13	1	3	1	10	0
2001	11	1	2	0	9	1
2002	8	1	1	0	7	1
2003	7	0	2	0	5	0
2004	9	4	1	0	8	4
2005	7	2	2	2	5	0
2006	7	1	0	1	7	0
2007	7	0	0	0	7	0
2008	5	0	1	0	4	0
2009	5	2	1	0	4	2
2010	4	0	0	0	4	0
2011	5	1	1	0	4	1
Total	88	13	14	4	74	9

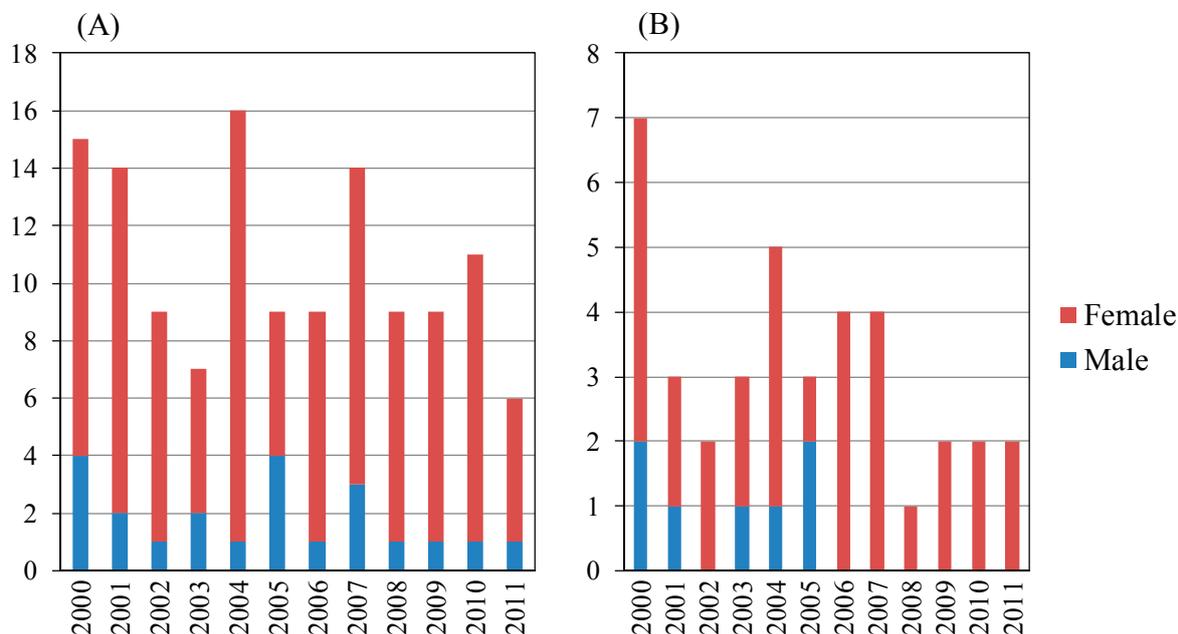
  

<b>(B)</b>						
	<b>Total patients (n = 101)</b>		<b>Male (n = 18)</b>		<b>Female (n = 83)</b>	
Year	A1	A2 or A3	A1	A2 or A3	A1	A2 or A3
2000	3	11	0	4	3	7
2001	3	9	0	2	3	7
2002	1	8	0	1	1	7
2003	1	6	0	2	1	4
2004	3	10	0	1	3	9
2005	1	8	0	4	1	4
2006	1	7	0	1	1	6
2007	1	6	0	0	1	6
2008	3	2	0	1	3	1
2009	2	5	0	1	2	4
2010	2	2	0	0	2	2
2011	1	5	0	1	1	4
Total	22	79	0	18	22	61

### 3.5. Clinical Features of Older Patients with AIH

AIH patients aged <65 years and  $\geq 65$  years numbered 57 and 36, respectively (Figure 3 and Table 4). Among those aged <65 years, fibrosis stage 1–3 and 4 patients numbered 51 and six, respectively, and among those aged  $\geq 65$  years, fibrosis stage 1–3 and 4 patients numbered 30 and six, respectively. The proportion of cirrhotic (F4) patients with AIH aged  $\geq 65$  years was the same in those aged <65 years (not statistically significant) (Table 4A). The 12 cirrhotic patients with AIH are shown in Table 2B. We identified six older cirrhotic patients with AIH, but three of them were male (Table 2B).

**Figure 3.** Annual numbers of autoimmune hepatitis patients with liver biopsy in the present study. (A) Total patients ( $n = 128$ ); male: female = 22:106; (B) Age  $\geq 65$  (years) ( $n = 38$ ), male: female = 7:31.



**Table 4.** Histopathological findings of the liver in autoimmune hepatitis according to age in the present study. (A) Staging of fibrosis; (B) Grading of inflammatory activity.

Year	(A)		(B)	
	Age < 65 (years)		Age $\geq 65$ (years)	
	F1-F3	F4	F1-F3	F4
2000	7	0	6	1
2001	8	1	3	0
2002	6	1	2	0
2003	4	0	3	0
2004	5	2	4	1
2005	6	0	1	2
2006	3	1	4	0
2007	2	0	3	0
2008	2	0	1	0
2009	2	0	0	2
2010	3	0	1	0
2011	3	1	2	0
Total	51	6	30	6

Table 4. Cont.

(B)				
Year	Age < 65 (years)		Age ≥ 65 (years)	
	A1	A2 or A3	A1	A2 or A3
2000	3	4	0	7
2001	2	7	1	2
2002	1	6	0	2
2003	0	4	1	2
2004	3	4	0	5
2005	1	5	0	3
2006	1	3	0	4
2007	0	2	1	2
2008	2	0	1	0
2009	1	1	1	1
2010	1	2	1	0
2011	1	3	0	2
Total	16	41	6	30

Among AIH patients aged < 65 years, patients with inflammatory activity grades A1 or A2 and A3 numbered 16 and 41, respectively. Among AIH patients aged ≥65 years, patients with inflammatory activity grades A1 or A2, and A3 numbered six and 30, respectively. The proportions of A2 and A3 patients with AIH aged ≥65 years were similar to those aged <65 years (not statistically significant) (Table 4B).

#### 4. Discussion

In the present study, recent trends for patients with autoimmune liver diseases and liver biopsies performed at a university hospital located in an urban area of Japan were investigated. Among the patients with PBC or AIH, at least 25.2% or 29.7%, respectively, were ≥65 years old. This can likely be attributed to the fact that the opportunity to diagnose and treat older people suffering from autoimmune liver diseases has increased in line with the aging of society in Japan. As a result of the development of non-invasive serum markers of hepatic fibrosis and transient elastography [25–27], with a concomitant decrease in occasions for liver biopsy, these numbers might in fact be underestimated. Kim *et al.* [2] reported that, in the United States, the peak incidence of patient age with PBC was 60–69 years for men and 70–79 years for women. More recently, Japanese national surveillance [28] reported that the peak incidence of patient age with PBC was 60–69 years for men and 50–59 years for women, regardless of asymptomatic or symptomatic PBC.

In the present study, we demonstrated that the proportion of PBC Scheuer stage 4 patients aged ≥65 years tended to be higher, and that all our six patients with stage 4 were female (Table 2A). Harada *et al.* [29] reported that the histological stage at the time of HCC diagnosis was an independent risk factor for HCC in females and that the time between the diagnosis of PBC and that of HCC was significantly shorter in males than in females. Attention should be paid to older PBC patients with advanced fibrosis.

For AIH patients, it seems that aging has no effect on stage, indicating that this disease can appear at any age, and could be classified into three different types of AIH [30]. Miyake *et al.* [31] reported that there are no apparent differences in the characteristics of AIH between older and younger patients, supporting the results of the present study. Although we identified six older cirrhotic patients with AIH, three of them were males. Thus, AIH is not just a severe female disease. Among older patients with AIH, confirming the accuracy of information about the disease seems important. Interestingly, we discovered that the proportion of AIH patients with moderate or severe activity (A2 or A3) is higher for males than for females (Table 3), indicating that among male patients with liver dysfunction of unknown etiology, AIH should be considered a possible etiology of their liver diseases. Liver biopsies could be useful in male patients with liver dysfunction of unknown etiology. It was reported that an androgenic/anabolic steroid could induce toxic hepatitis [32]. Gender differences in the proportion of AIH patients with moderate or severe activity (A2 or A3) might be caused by the effects of sex steroid hormone receptors such as androgen receptors [33,34]. Further studies will be needed. The number of female patients with PBC or AIH was greater than male patients, and this may reflect the population of these autoimmune liver diseases. In spite of this tendency, the number of older cirrhotic patients with AIH was equally split between males and females (Table 2B). This may be due to the fact that male patients with AIH tend to have a more severe stage than females, or that it might be difficult to diagnose male patients with AIH using the present criteria [35,36]. Special attention should also be paid to the diagnosis and/or treatment of older male patients with AIH. In such cases, liver biopsies could be useful, and older patients with autoimmune liver diseases may have more severe cases than expected.

In Japan, HCC developed in 5.1% of patients with AIH, and cirrhosis was commonly found in elderly individuals [37]. Ohira *et al.* [37] reported that the mean age at diagnosis of HCC was 69 years, and that the male-to-female ratio was 1:5.7. AIH patients with liver cirrhosis are at high risk for HCC. Although diagnosis of AIH has recently been performed using the AIH scoring system [35,36] in consideration of the possibility of other liver diseases, other fibrogenetic factors such as non-alcoholic fatty liver diseases and alcohol intake should also be considered [36,38]. In the present study, we found 12 cirrhotic patients with AIH (Table 2); only one patient revealed alcohol intake, but four patients were overweight or obese. In Japanese cirrhotic AIH patients, these fibrogenetic factors might have partly contributed to this condition.

Previous studies have established that PBC is associated with HLA-DR8 [39] and six non-HLA loci: *IL12A*, *IL12RB2*, *IRF/TNPO3*, *ORMDL3/IKZF3*, *MMEL1*, and *SP1B* [40]. Mells *et al.* [40] reported that *STAT4*, *DENND1B*, *CD80*, *IL7R*, *CXCR5*, *TNFRSF16F*, and *NFKB1* were new candidate genes associated with the genetic risk factors for PBC. A recent genome-wide association study revealed *TNFSF15* and *POU2AF1* as susceptibility loci for PBC in the Japanese population [41]. Another genome-wide association study revealed that AIH type 1 was associated with variants in the major histocompatibility complex region, and identified the variants *SH2B3* and *CARD10* as likely risk factors [42].

In PBC patients, the aspartate aminotransferase-to-platelet ratio index (APRI) was significantly correlated with the histological degree of liver fibrosis, with a limited value of scores between 1.0 and 1.5 [43]. Although it was reported that APRI appeared to be of no value in AIH patients [44], serum surrogate markers such as hyaluronic acid level, type 4 collagen level, and APRI, as well as liver stiffness measurement, are helpful in predicting significant fibrosis of the liver in non-viral liver diseases [25]. In the near future, we will not have to perform a liver biopsy to diagnose PBC and AIH.

Further studies of the differences between AIH pathology and PBC pathology might shed some new light on better management of these diseases.

In Japan, opportunities to manage patients with autoimmune liver diseases such as AIH and PBC have been on the increase. In conclusion, we should pay particular attention to the progression of fibrosis and inflammatory activity of the liver when we treat older patients suffering from autoimmune liver diseases. For this reason, at the present time, liver biopsy is recommended for obtaining accurate information.

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### Author Contributions

Yuki Haga and Tatsuo Kanda collected and analyzed data, saw the patients, and contributed to the writing and editing of the manuscript. Katsuhiko Hagiwara collected and helped analyze data. Reina Sasaki, Masato Nakamura, Shin Yasui, Makoto Arai, and Osamu Yokosuka saw the patients. Xia Jiang, Shuang Wu, and Shingo Nakamoto collected and analyzed data and helped edit the manuscript.

### Conflicts of Interest

The authors declare no conflict of interest.

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