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Clinical Significance of Immunoglobulin G4-Associated Autoimmune Hepatitis

Short title: Clinical significance of IgG4-AIH

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ABSTRACT

Background Immunoglobulin (Ig) G4-associated autoimmune hepatitis (AIH) is a recently identified and possibly new disease entity. However, the epidemiology and clinical features of IgG4-associated AIH remain uncertain. The aim of this study was to determine the prevalence and the clinical, serological, and histological characteristics of IgG4-associated AIH.

Methods We examined the clinical features, serum IgG4 concentration, liver biopsy histology, and IgG4-bearing plasma cell infiltration of 60 patients with type 1 AIH and 22 patients with autoimmune pancreatitis.

Results High serum IgG4 concentration (≥ 135 mg/dL) and IgG4-bearing plasma cell infiltration in the liver (≥ 10/HPFs) were found in 2 of 60 (3.3%) cases with type 1 AIH. These patients had high serum levels of IgE, giant cell change, and rosette formation in the liver. Although corticosteroid therapy ameliorated serum IgG4 concentration and liver enzymes and histology, one case developed IgG4-related sclerosing cholangitis after 5 years of follow-up.

Conclusions Since IgG4-associated AIH was found in over 3% of Japanese patients with type 1 AIH in our cohort, further studies are needed on this possible new disease entity and its impact on the diagnostic guidelines of AIH.

Key words: autoimmune hepatitis, autoimmune pancreatitis, IgG4, histology, sclerosing cholagitis

Introduction

Autoimmune hepatitis (AIH) is an organ-specific autoimmune disease characterized by chronic inflammation of the liver, elevated transaminase levels, hypergammaglobulinemia, serum autoantibodies, histologic evidence of interface hepatitis, and a favorable response to immunosuppressive treatment (1-3). Type 1 AIH is the major form of AIH in Japanese and Caucasoid adults, and can be distinguished by the presence of circulating anti-nuclear antibodies (ANA) and/or anti-smooth muscle antibodies (SMA). The diagnosis of type 1 AIH is based on the revised scoring system developed by the International Autoimmune Hepatitis Group (IAIHG) (4).

We recently reported a case suggesting the existence of a new disease entity termed Immunoglobulin G4 (IgG4)-associated AIH (5). Although the revised IAIHG disease score of this patient was 18 and representative of definite AIH, a high serum IgG4 concentration before administration of corticosteroid therapy was detected. Moreover, immunostaining of liver tissues prior to treatment showed abundant plasma cells with strong immunohistochemical reactivity to IgG4. Abdominal computed tomography, endoscopic retrograde cholangiography, and magnetic resonance cholangiopancreatography showed no abnormalities of the extra bile ducts or pancreas. Raised serum IgG4 concentration and IgG4-bearing plasma cell infiltration have a high sensitivity and specificity for the diagnosis of autoimmune pancreatitis (AIP) (6-8) and IgG4-related sclerosing cholangitis or IgG4-associated cholangitits (9-10). Thus, we suggested that IgG4-associated AIH was in fact a IgG4-related disease (5). Since then, the epidemiology and clinical significance of IgG4-associated AIH have remained largely unknown. Chung *et al.* recently conducted IgG4 immunostaining of liver biopsies from 26 patients with AIH (11). Although they reported that 9 out of 26 patients had more than 5 IgG4-positive plasma cells infiltration in the liver, no cases showed high serum IgG4 in their cohort. We have also found that patients with AIP had histological liver findings that included portal inflammation, large bile duct damage, portal sclerosis, lobular hepatitis, and cholestasis and/or IgG4-bearing plasma cell infiltration in the liver, and have accordingly proposed that histological liver changes in AIP be considered as an IgG4 hepatopathy as well (8). In the present study, we investigated serum IgG4 concentrations and IgG4 immunostaining of the liver in patients with type 1 AIH, compared them with

those in AIP, and proposed diagnostic criteria for IgG4-associated AIH.

METHODS

Subjects

Between June 1985 and December 2006, 70 consecutively-treated type 1 AIH patients (12 men and 58 women; median age 57 years [interquartile range, (IQR) 45 – 63 years]) were seen at Shinshu University Hospital, Japan. All patients were diagnosed as having probable or definite AIH according to the scoring system from the IAIHG. Among them, 60 (86%) subjects from whom serum and paraffin-embedded liver biopsy samples before administration of corticosteroids were available were registered for this study and summarized in Table 1. No patients had a pancreatic mass or common bile duct strictures detectable by ultrasonography, computed tomography, and/or magnetic resonance imaging. All 60 patients were treated with corticosteroids, and none were given azathioprine during follow-up. All patients were negative for hepatitis B surface antigen, antibody to hepatitis B core antigen, antibody to hepatitis C virus, and antibody to human immunodeficiency virus. No patients were positive for antimitochondrial antibody–M2. No patients had potential drug induced liver injury. Twenty-two patients with AIP were enrolled as control cases. All were biopsied. The diagnosis of AIP was based on criteria released by the Japan Pancreas Society (12). Serum samples were obtained at the time of liver biopsy and stored at -70°C until testing. The protocol of this study conformed to the Declaration of Helsinki, and was approved by the ethics committee of Shinshu University School of Medicine.

Laboratory Testing

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and other relevant biochemical tests were performed using standard methods (13). ANA and SMA were determined by indirect immunofluorescence on murine tissue sections as reported previously (8). A serum titer of 1:40 or greater was considered positive for ANA and SMA. Patterns of ANA reactivity were also recorded. Anti-double-stranded DNA (< 12 IU/mL), anti-Ro (SS-A), anti-La (SS-B), and antimitochondrial antibody–M2 were tested by ELISA as reported previously (8). Serum IgG4 concentrations were determined by single

radial immunodiffusion (IgG4-SD RID kit, RN109.3, Bindng Site, Birmingham, UK) as reported previously (6) or by a nephelometric assay (IgG4 BN™II kit, NK009.T, Bindng Site, Birmingham, UK) (normal value < 135 mg/dL).

Genomic DNA from patients was isolated by phenolic extraction of sodium dodecyl sulfate-lysed and proteinase K-treated cells as described previously (14). HLA class I and II alleles were determined using a Micro SSPTM DNA Typing Kit (One Lamda, Canoga Park, CA). DNA typing of DRB1 and DQB1 alleles was performed by polymerase chain reaction-restriction fragment length polymorphism analysis as previously described (14-15).

Histological Evaluation and Immunohistochemistry of IgG and IgG4

Liver biopsies were performed by percutaneous sampling of the right lobe with a 14-gauge needle in all but one patient, whose liver specimen was obtained during surgery. All biopsies were 1.5 cm or more in length. Liver specimens were taken before administration of corticosteroid therapy in 60 patients with AIH and 22 patients with AIP. Formalin-fixed and paraffin-embedded specimens were prepared and used for histopathological and immunohistochemical studies. Sections measuring 4 μm were cut from each paraffin block and stained with hematoxylin and eosin, periodic acid-Schiff after diastase digestion, Azan-Mallory, silver impregnation reticulin, or silver impregnation orcein, with the remaining material being used for immunohistochemical analysis. The following 11 histological features were assessed by two experienced pathologists (Y.Z. and Y.N.), who analyzed each feature under code and independently of other data: (1) portal fibrosis (0, absent; 1, periportal fibrosis; 2, bridging fibrosis; 3, bridging fibrosis with lobular distortion; 4, cirrhosis); (2) portal inflammation (0, absent; 1, inflammation in ≤ 1/3 of periportal areas; 2, inflammation in 1/3 to 2/3 of periportal areas; 3, inflammation in ≥ 2/3 of periportal areas); (3) interface hepatitis (0, absent; 1, interface hepatitis in ≤ 1/3 of periportal areas; 2, interface hepatitis in 1/3 to 2/3 of periportal areas; 3, interface hepatitis in ≥ 2/3 of periportal areas); (4) lobular hepatitis (1, 0-2 focal necrosis / high power field (HPF); 2, ≥ 3 focal necrosis / HPF; 3, zonal necrosis); (5) plasma cell infiltration (1, 0-9 cells / HPF in portal area; 2, 10-19 cells / HPF; 3, ≥ 20 cells / HPF); (6) eosinophil infiltration (0, 0-4 cells / HPF in portal area; 1, ≥ 5 cells / HPF); (7)

syncytial giant cell change (0, absent; 1, present); (8) rosette formation (0, absent; 1, present); (9) bile duct loss (0, absent; 1, present); (10) bile duct damage (-, absent; +, irregularity of cellular and nuclear arrangement of the biliary epithelium with or without narrowing of the bile duct lumen); and (11) cholangitis (0, absent; 1, present).

Immunostaining for IgG and IgG4 was performed using mouse monoclonal antibodies against human IgG (Dako Cytomation, Glostrup, Denmark), and human IgG4 (ZYMED Laboratory, San Francisco, Calif.) as reported previously (9). Counts were tallied for IgG- and IgG4-bearing plasma cells per HPF, and the number of positive cells was expressed as the mean of triplicates and compared. The ratio of IgG4-positive to IgG-positive cells was also calculated for each case.

Statistical analysis

The Mann-Whitney *U* test was used to analyze continuous variables where appropriate. The chi-square test with Yates's correction was used for analysis of categorical data. In cases where the number of subjects was less than 5, Fisher's exact test was used. A $P \leq 0.05$ was considered to be significant. Statistical analysis was performed using SPSS software (version 15.0J; SPSS, Chicago, IL).

RESULTS

Clinical Characteristics of Patients

The clinical profile of the experimental patient cohort is shown in Table 1. Forty-nine (82%) were women, and the median age at presentation was 58 years. All patients were graded by the IAIHG scoring system prior to treatment. Of them, 48 (80%) patients satisfied the criteria for a definite diagnosis of AIH and the remaining 12 patients met the requirements for a probable diagnosis. Although no AIP patients fulfilled the definite criteria for AIH, 11 of 22 (50%) patients met the probable criteria for AIH as well. The median IAIHG score was significantly higher in patients with AIH than in those with AIP. AIH patients also had significantly higher median serum levels of ALT, AST, and total bilirubin. There were significant differences in positivity for ANA and SMA in AIH vs. AIP, but the prevalence of HLA DR4 did not differ between the two groups.

Median serum IgG4 concentration, IgG4:IgG ratio, and serum IgE level were

significantly higher in AIP patients than in cases with AIH. However, an elevated serum IgG4 concentration (≥ 135 mg/dL) was detected in 4 of 60 (6.7%) patients with AIH, and the serum IgG4 concentration and IgG4:IgG ratio in each of these patients were 146, 215, 557, and 642 mg/dL and 0.041, 0.072, 0.232, and 0.114, respectively. We previously performed receiver operating characteristic curve analysis to determine the optimal cutoff value of serum IgG4:IgG ratio to best differentiate patients with AIP from other diseases.(8) This value was 0.073, meaning 2 of 60 (3.3%) AIH patients had an extraordinarily high serum IgG4 concentration and IgG4:IgG ratio.

Histopathology of Liver Biopsies in Patients with AIH and AIP

As shown in Table 2, portal and periportal fibrosis was present in all 60 patients with AIH, and 33 of them (55%) showed bridging fibrosis. Twenty-two patients (37%) had cirrhosis. Portal inflammation was evident in all but one patient, and 55 (92%) were also associated with interface hepatitis. Lobular hepatitis, which was defined as focal necrosis ≥ 3/HPF, was found in 39 patients (65%). Marked plasma cell infiltration (≥ 20/HPF) and eosinophil infiltration (≥ 5/HPF) in portal tracts were present in 30 (50%) and 18 patients (30%), respectively. Giant cell change and rosette formation were found in 7 (12%) and 9 patients (15%), respectively. Only 3 patients had both giant cell change and rosette formation. Bile duct damage was found in 10 patients (17%), but chronic nonsuppurative destructive cholangitis and bile duct loss were not found in any of the cases.

Immunostaining for IgG and IgG4 demonstrated many IgG- and IgG4-bearing plasma cells in the portal tract. The number of IgG4-bearing plasma cells in patients with AIP (6.0/HPF; IQR, 2.3-12.3) was significantly higher than in patients with AIH (0.0/HPF; IQR, 0.0-3.8; $P < 0.001$), but the number of IgG-bearing plasma cells were not. More than 5 IgG4-positive cells (/HPF) were found in 5 of 60 (8%) patients with AIH. The IgG4-positive cells and ratio of IgG4:IgG bearing plasma cells in each of these patients were 6, 6, 7, 24, and 29/HPF and 0.082, 0.102, 0.071, 0.282, and 0.528, respectively. The latter 2 patients were also the cases with elevated serum IgG4 and IgG4:IgG ratio.

All patients with AIP in this study had histological liver findings and/or IgG4-bearing plasma cell infiltration in the liver, and were thus considered to have an IgG4 hepatopathy.

Clinical Significance of IgG4-Associated Autoimmune Hepatitis

In our cohort, high serum IgG4 concentration and more than 10 IgG4-positive cells (/HPF) were found in 2 patients. As shown in Table 1, the 2 patients with IgG4-associated AIH had higher median serum levels of IgG4, IgG4:IgG ratio, and IgE compared with the 58 cases of classical AIH. The HLA DRB1*0405 allele was found in one patient with IgG4-associated AIH. Liver biopsies of the 2 patients showed similar chronic active hepatitis with bridging fibrosis. Hepatic activity was high, and both patients showed interface hepatitis and zonal necrosis. Interestingly, giant cell change and rosette formation were simultaneously observed in both patients with IgG4-associated AIH (Table 2). The median numbers of IgG- and IgG4-bearing plasma cells in patients with IgG4-associated AIH (70.3/HPF and 26.5/HPF) tended to be higher than those for classical AIH (24.5/HPF and 0.0/HPF) and AIP (14.5/HPF and 6.0/HPF).

Clinical Course of IgG4-associated Autoimmune Hepatitis

Case 1 in this study was previously reported as the first proposed case of IgG4-associated AIH (5). The patient's serum IgG4 concentrations and liver enzymes eventually normalized with continuous low dose corticosteroid therapy (5.0 – 10.0 mg/day). However, after 5 years of follow-up, her serum alkaline phosphatase and γ-glutamyl transpeptidase rose to 622 IU/L and 936 IU/L, respectively. Although no abnormalities of the bile duct were found before administration of corticosteroid therapy, distal and proximal biliary strictures became evident by endoscopic retrograde cholangiography (Figure 1) and magnetic resonance cholangiopancreatography. In addition, abundant IgG4-bearing plasma cell infiltration was found in a biopsy of the common bile duct, despite serum IgG4 concentrations remaining normal at 75 mg/dL. Hence, she was the first patient diagnosed with IgG4-associated AIH who later developed IgG4-related sclerosing cholangitis.

Case 2 was a 42-year old man who was admitted to our hospital because of elevated liver enzymes (16). Serum IgG and IgE concentrations prior to corticosteroid therapy were extremely high. His ANA antibody, anti-double-strand DNA, and SMA were all positive. A first liver biopsy showed changes associated with typical AIH: interface

hepatitis, lobular hepatitis, rosette formation, syncytial multinucleated giant cell change, and marked plasma cell infiltration. No biliary epithelial changes were found. Imaging modalities showed no abnormalities of the extrahepatic bile ducts or pancreas. A serum IgG4 concentration of 642 mg/dL was detected in stored serum, and immunostaining of liver tissue showed abundant plasma cells with strong immunohistochemical reactivity to IgG4. He fulfilled the criteria for definite AIH and was administered corticosteroids at 60 mg/day. Four weeks later, serum IgG4 and IgE concentrations were decreased to 452 mg/dL and 909 IU/L, respectively. Although a second liver biopsy performed 7 months after the first showed remaining portal sclerosis, almost all other histological findings were improved except for mild portal inflammation. Serum IgG4 and IgE concentrations were normalized. Furthermore, IgG4-bearing plasma cell infiltration in the liver was absent (0/HPF). After 13 years of follow-up, his transaminases are still elevated but below 100 IU/L with continuous low dose corticosteroid therapy (5.0 mg/day), and image modalities show no abnormalities. His ANA antibody titer (1:1280) and anti-double-strand DNA (>400 IU/mL) were still abnormal.

DISCUSSION

In an earlier report, a strong and unexpected association was seen between serum IgG4 concentration and IgG4-bearing plasma cell infiltration in the liver in one case of type 1 AIH, raising the possibility of a new disease entity termed IgG4-associated AIH (5). In the present study, we investigated serum IgG4 concentration and IgG4 immunostaining of the liver in 60 Japanese patients with type 1 AIH and looked for correlations with liver histology and clinical features in comparison with AIP.

Based on our findings, we have provisionally set the diagnostic criteria for IgG4-associated AIH as follows: 1) having definite AIH according to the IAIHG scoring system, 2) serum IgG4 concentration ≥ 135 mg/dL, and 3) immunostaining of IgG4 showing ≥ 10/HPF IgG4-bearing plasma cell infiltration in the portal tract. We ultimately identified 2 patients (3.3%) who fulfilled these criteria among our AIH cases. This rate was quite low, as we had expected. Conversely, Chung et al. recently reported that 9 of 26 patients with AIH showed more than 5 IgG4-positive plasma cells (/HPF) in the liver (11). Although serum IgG4 concentration in all patients was normal at less than 80 mg/dL,

these cases were labeled as having IgG4-associated AIH and thus distinct from IgG4-related disease. In general, the concept of an IgG4-related disease is high serum IgG4 concentration and abundant IgG4-bearing plasma cell infiltration in the affected organs. Although it is possible that several patients had IgG4-positive cell infiltration without high serum IgG4 concentration, it is difficult to draw the conclusion that all of their cases can be classified as having an IgG4-associated disease. Koyabu et al. recently reported that an IgG4/IgG1-bearing plasma cell ratio of > 1 in the liver is specific for IgG4-related diseases.(17) In our 2 cases, the ratio of IgG4/IgG1-bearing plasma cell in the liver was > 1. Hence, this ratio might be useful marker to be diagnosed as IgG4-related diseases.

We recently reported on IgG4 hepatopathy in AIP patients because almost all cases had liver dysfunction and histological changes, such as interface and lobular hepatitis (8). Since our patients with IgG4-associated AIH had high serum IgG4 and abundant IgG4-bearing plasma cell infiltration in the liver, we believe this disease entity should be considered as an IgG4-associated disease (IgG4 hepatopathy) rather than AIH. Using the IAIHG scoring system, 50% of our 22 AIP patients had probable AIH. Hence, we excluded all AIH cases listed as probable for a more precise evaluation of IgG4-associated AIH. We also selected 135 mg/dL as the cut-off value for serum IgG4 because of its high accuracy of AIP diagnosis (6). Since IgG4-bearing plasma cell infiltration is a characteristic finding in AIP, (6, 18) IgG4-related sclerosing cholangitis, and IgG4-associated cholangitis,(9-10) we included the criteria of more than 10 IgG4-bearing plasma cell infiltration (/HPF) in the liver as well. Moreover, we believe that AIH patients with pancreatic abnormalities should be excluded because a prior study showed that interface hepatitis was found in 24% of patients with AIP (8). Although all AIP patients had histological liver changes and/or IgG4-bearing plasma cell infiltration in the liver, no patients fulfilled the criteria for definite AIH. Hence, they were considered to have AIP with histological damage in the liver, not IgG4-associated AIH. A relatively small number of patients with AIH were studied in this report since patients having both stored sera and paraffin-embedded liver biopsy samples are scarce. As such, a larger sample group will be needed to compare IgG4-associated AIH with classical AIH.

IgG4-related diseases are primarily found in the pancreatobiliary system and

hepatic parenchyma (6-9). Our cases were mainly affected in the liver and resembled AIH both clinically and pathologically (5, 16). The difference between IgG4-associated AIH and comorbid hepatic injury in AIP are considered to be: 1) patients with IgG4-associated AIH have a much higher degree of IgG4-bearing plasma cell infiltration in the liver compared not only with classical AIH, but also AIP; 2) giant cell change and rosette formation are obvious; and 3) bile duct damage or loss is not found. Taken together, it appears that the histological liver findings of IgG4-associated AIH seem to be more severe compared with classical AIH and AIP.

Interestingly, one patient with IgG4-associated AIH who was administered low dose corticosteroid therapy developed IgG4-related sclerosing cholangitis after 5 years of follow-up. Since we did not perform a bile duct biopsy prior to treatment, we can not exclude the possibility that she had sclerosing cholangitis at that time. However, no bile duct abnormalities were seen by imaging modalities or in liver biopsy samples before therapy. AIH/primary sclerosing cholangitis overlap syndrome has been reported with increasing incidence (19-24). Although these studies did not examine serum IgG4 concentration or IgG4-immunostaining of liver biopsies, it is possible that a case of IgG4-associated AIH with sclerosing cholangitis might have been included in their populations.

The fundamental nature of IgG4-related diseases is enigmatic. Since IgG4 and IgE immune responses to antigens often occur in allergic disorders (25-26), damage to pancreatic acinar cells, cholangiocytes, and hepatocytes via an allergic type of reaction to target tissue cells initially induced by a dietary or bacterial antigen is plausible. In addition, Zen and Nakanuma, who are coauthors in this study, recently reported that IgG4-related diseases were characterized by the over-production of T helper 2 and regulatory cytokines, both of which are closely involved in the pathogenesis of allergic disorders (27). Patients with IgG4-associated AIH had high serum IgG4 and IgE concentrations and lobular hepatitis with marked IgG4-bearing plasma cell infiltration, suggesting that an allergic reaction to hepatocytes or molecules in liver parenchyma might be a possible pathogenesis of this disease. However, such a notion is highly speculative and would require additional in-depth studies to show mast cell involvement in liver tissues of AIP and IgG4-associated AIH patients. At present, we can only describe

the observation of high serum IgE concentrations and cannot provide a sound scientific basis for its occurrence.

Susceptibility of AIH and AIP are influenced by genetic factors, specific HLA alleles, amino acid sequences at the presentation site of HLA molecule,(28-29) and Fc receptor-like gene 3 and cytotoxic T-lymphocyte antigen 4 single nucleotide polymorphisms(28, 30-33). Those genetic markers should be investigated to our 2 patients.

In conclusion, IgG4-associated AIH was found in over 3% of classical AIH cases in Japan and is characterized by high serum IgG4 and IgE concentrations and IgG4-bearing plasma cell infiltration in the liver. Since IgG4-associated AIH is clearly an IgG4 hepatopathy, this disease should be differentiated from classical AIH. Further studies are needed to clarify the epidemiology and pathogenesis of this possible new disease entity.

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Table 1. Demographic and Clinical Characteristics of AIH and AIP Patients

Characteristics	AIH (n = 60)	AIP (n = 22)	*P* value	AIH	
				IgG4-AIH (n = 2)	Classical AIH (n = 58)
Median age, yrs (IQR)	58 (46-64)	63 (57-66)	0.06	48 (42-54)	58 (46-65)
Female, n (%)	49 (82)	3 (14)	<0.001	1 (50)	48 (83)
AIH score, median (IQR)	17 (16-19)	9 (6-10)	<0.001	17 (16-19)	17 (16-19)
Definite, n (%)	48 (80)	0 (0)	<0.001	2 (100)	46 (79)
Probable, n (%)	12 (20)	11 (50)		0 (0)	12 (21)
Median values (IQR)					
ALT (n.v. 7-45 IU/L)	573 (153-1090)	66 (27-140)	<0.001	497 (253-739)	573 (150-1103)
AST (n.v. 12-37 IU/L)	437 (150-907)	36 (24-59)	<0.001	425 (199-650)	437 (145-907)
ALP (n.v. 124-367 IU/L)	418 (295-548)	481 (322-753)	0.429	539 (472-605)	401 (294-507)
Bilirubin (n.v. 0.3-1.2 mg/dL)	2.2 (1.0-8.5)	1.1 (0.6-1.8)	0.002	5.6 (2.5-8.6)	2.1 (1.0-8.2)
Alb (n.v. 4.2-5.1 g/dL)	3.6 (3.0-3.9)	3.7 (3.4-4.0)	0.314	3.0 (2.8-3.2)	3.6 (3.1-3.9)
IgG (n.v. 870-1700 mg/dL)	2940 (2330-3493)	2494 (1598-3268)	0.096	4015 (2403-5627)	2940 (2328-3464)
IgG4 (n.v. <135 mg/dL)	23 (18-50)	699 (346-1335)	<0.001	560 (557-642)	22 (18-47)
IgG4:IgG	0.009 (0.006-0.015)	0.253 (0.189-0.410)	<0.001	0.173 (0.114-0.232)	0.009 (0.006-0.014)
IgE (n.v. <361 IU/mL)	64 (20-381)	246 (105-700)	0.010	1490 (1330-1650)	58 (18-306)
High-IgG4 levels, n (%)	4 (7)	21 (95)	<0.001	2 (100)	2 (3)

ANA-positive, n (%)	60 (100)	17 (77)	<0.001	2 (100)	58 (100)
SMA-positive, n (%)	30/54 (56)	0 (0)	<0.001	1 (50)	29/52 (56)
ds-DNA-positive, n (%)	4 (7)	1 (5)	0.593	1 (50)	3 (5)
SS-A-positive, n (%)	5 (8)	0 (0)	0.200	0 (0)	5 (9)
SS-B-positive, n (%)	1 (2)	0 (0)	0.732	0 (0)	1 (2)
HLA-DR4, n (%)	38/56 (68)	9/15 (60)	0.557	1 (50)	37/54 (69)

Abbreviations: AIH; autoimmune hepatitis, AIP; autoimmune pancreatitis, IgG4-AIH; IgG4-associated autoimmune hepatitis, IQR: interquartile range; n.v., normal value; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; γGTP, γ-glutamyltransferase; ANA, anti-nuclear antibody; SMA, anti-smooth muscle antibody; ds-DNA, anti-double stranded DNA.

Table 2. Histological Characteristics of Patients with AIH and IgG4-AIH

	AIH (n = 60)	IgG4-AIH (n = 2)	Classical AIH (n = 58)
Bridging Fibrosis	33 (55%)	2 (100%)	31 (54%)
Cirrhosis	22 (37%)	0 (0%)	22 (38%)
Portal Inflammation (≥2/3 of periportal areas)	43 (72%)	2 (100%)	41 (71%)
Interface Hepatitis (≥2/3 of periportal areas)	23 (38%)	2 (100%)	21 (36%)
Lobular Hepatitis (zonal necrosis)	15 (25%)	2 (100%)	13 (22%)
Plasma Cells (≥ 20 / HPF)	20 (33%)	2 (100%)	18 (31%)
Eosinophils (≥ 5 / HPF)	18 (30%)	1 (50%)	17 (29%)
Giant Cell Change	7 (12%)	2 (100%)	5 (9%)
Rosette Formation	9 (15%)	2 (100%)	7 (12%)
Bile Duct Damage	10 (17%)	0 (0%)	10 (17%)

Abbreviations: AIH; autoimmune hepatitis, IgG4-AIH; IgG4-associated autoimmune hepatitis

Figure Legends

Figure 1. Endoscopic retrograde cholangiography after 5 years of follow-up showing smooth narrowing of the common bile duct and intrahepatic biliary strictures