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Anti-mitochondrial M2 antibody-positive autoimmune hepatitis

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Abstract. Anti-mitochondrial M2 antibody (AMA-M2) is specific to primary biliary cirrhosis (PBC), but can also be found in certain patients with autoimmune hepatitis (AIH). Effective methods of differentiating between PBC and AIH are required, as their clinical course and management are different. Titers of AMA-M2 were analyzed before and after follow-up in patients with PBC or AIH. Patients who underwent liver biopsy and were diagnosed with either AIH (10 patients) or PBC (3 patients) were enrolled in the study. The AMA-M2 antibody titers of these patients were analyzed upon hospital admission. AMA-M2 reacted with the pyruvate dehydrogenase complex-E2, branched-chain 2-oxo acid dehydrogenase complex and 2-oxoglutaric acid dehydrogenase complex in the assay utilized for this study. The cut-off value for AMA-M2 was 5. Six AIH patients were AMA-M2(-) and 4 were AMA-M2(+). The titer for the AIH patients who were AMA-M2(+) was 24.8±14.8, compared with 324±174 in the patients with PBC (P=0.0138). Three AMA-M2(+) AIH patients were followed-up after liver biopsy. The AMA-M2 levels had decreased in all 3 patients, becoming undetectable in 2 of them. In conclusion, certain patients with AIH in this study were found to be AMA-M2(+), but the titers were significantly lower than those in the patients with PBC. At follow-up, the AIH patients exhibited decreased AMA-M2 titers.

Introduction

Autoimmune hepatitis (AIH) is caused by an immune attack on the hepatocytes (1). Patients with AIH are positive for auto-antibodies, such as anti-nuclear, anti-smooth muscle and liver-kidney microsomal type 1 antibodies (2). Chronic inflammation in the liver can progress to liver cirrhosis (3). One major problem of AIH is that an acute presentation or exacerbation of the disease can progress to liver failure (4,5). Furthermore, patients with AIH can be susceptible to hepatocellular carcinoma (6); therefore, patients should be followed-up for exacerbation and hepatocellular carcinoma (7). Primary biliary cirrhosis (PBC) is characterized by nonsuppurative cholangitis and autoimmune-mediated destruction of the small or medium-sized bile ducts (8). Inflammation causes cholestasis and the liver develops cirrhosis (9). AIH is treated with immunosuppressive drugs, such as prednisolone or azathioprine (10,11), while PBC is treated with ursodeoxycholic acid (10). It is important to differentiate AIH from PBC due to the differences in the clinical course and management of the two diseases.

Anti-mitochondrial antibody (AMA) can be tested via indirect immunofluorescence (12). In this technique, unfixed sections of the kidney and stomach of Wistar rats are used. AMA is used for the diagnosis of PBC (13), as 90-95% of patients with PBC are positive for AMA (14); however, the use of AMA as a differential test is problematic, as 5% of patients with AIH are positive for AMA (15), and the interpretation of a positive AMA result in AIH is difficult.

The auto-antigens of AMA have been identified as pyruvate dehydrogenase complex-E2 (PDC-E2), branched-chain 2-oxo acid dehydrogenase complex and 2-oxoglutaric acid dehydrogenase complex (16,17). The antibody to these antigens is known as AMA-M2 (18). AMA-M2 is more specific to PBC than AMA, and the determination of its titer is feasible (19). It is widely accepted that AMA-M2 is useful for the diagnosis of PBC, and a scoring system for PBC that uses the antibody has been generated (20); however, patients with AIH can still test positive for AMA-M2 (21).

The present study investigates AIH patients who are AMA-M2(+). Titers of AMA-M2 are compared between AMA-M2(+) AIH patients and PBC patients, and the changes in AMA-M2 during the follow-up of the patients with AIH are reported.

Patients and methods

Patients. The records of patients who underwent liver biopsy between April 2009 and March 2014 were retrospectively

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Table I. Blood analysis comparison between AMA-M2(+) AIH patients and PBC patients.

Parameter	AMA-M2(+) AIH patients	PBC patients	P-value
Age (years)	59.3±14.8	61.8±19.9	0.8467
ALP (IU/l)	457±126	780±349	0.1312
AST (IU/l)	631±212	59±22	0.1044
ALT (IU/l)	663±777	66±22	0.1752
γ-GTP (IU/l)	258±135	298±339	0.8340

One-way analysis of variance was applied to the results. AMA-M2, anti-mitochondrial M2 antibody; AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase.

reviewed. Patients were selected, as this study was retrospective. Patents were included only when they were diagnosed as AIH or PBC with liver biopsy, and their AMA-M2 was subsequently analyzed. Liver biopsies were crucial for the histological confirmation of the diagnosis. The liver biopsy was performed during hospitalization with an automatic liver biopsy needle, which measured 14 G in diameter and 150 mm in length (ACE-141501; TSK Laboratory, Tochigi, Japan) (22). Ten patients were diagnosed with AIH (2 men and 8 women), and 4 patients were diagnosed with PBC (all women). No patients were diagnosed with the PBC-AIH overlap syndrome. This study was reviewed by the ethics committee of the National Hospital Organization Shimoshizu Hospital (Yotsukaido, Japan) and was not considered to be a clinical trial, as it was performed as a part of routine clinical practice. The committee waived the requirement for informed consent due to the retrospective nature of the study. Patient anonymity was preserved.

Diagnosis of AIH. The diagnosis of AIH was based on a scoring system proposed by the International Autoimmune Hepatitis Group (23). AIH was diagnosed when the pre-treatment score was >10 or the post-treatment score was >12 and the pathological findings were consistent with AIH.

Diagnosis of PBC. The diagnosis of PBC was based on guidelines of the American Association for the Study of Liver Diseases (13). Patients were diagnosed with PBC when alkaline phosphatase (ALP) levels were elevated, the AMA test was positive and there was evidence of nonsuppurative cholangitis and the destruction of small or medium-sized bile ducts. The diagnosis of PBC-AIH overlap syndrome was based on the Paris criteria (24).

Laboratory data. The laboratory data analyzed in the present study were ALP, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP) and AMA-M2. The data were collected upon admission of the patients to the hospital. Detection of AMA-M2 was outsourced to the LSI Medience Corp. (Tokyo, Japan). AMA-M2 reacted with the PDC-E2, the branched-chain 2-oxo acid dehydrogenase complex and the 2-oxoglutaric acid dehydrogenase complex (16). The cut-off value for AMA-M2 was 5. Patients with values <5 were considered negative for AMA-M2; patients with values >5 were considered positive.

Statistical analysis. Results are presented as the mean ± standard deviation. One-way analysis of variance was performed for the analysis of the laboratory data using the statistical software JMP 10.0.2 (SAS Institute, Inc., Cary, NC, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Comparison of AMA-M2(+) AIH patients and PBC patients. The laboratory data of the AMA-M2(+) AIH patients and PBC patients were compared to confirm that the patients had different diseases (Table I). ALP levels tended to be higher in the patients with PBC than those in the AMA-M2(+) AIH patients. AST and ALT levels were higher in the AMA-M2(+) AIH patients than those in the PBC patients. No significant differences were found between the groups ($P>0.05$). Higher levels of ALP indicate bile duct obstruction, a characteristic of PBC (13). The higher AST and ALT levels in the AMA-M2(+) AIH patients were due to the acute presentation.

Titers of AMA-M2 were compared between the AMA-M2(+) AIH patients and the patients with PBC (Fig. 1). Titers of AMA-M2 were 24.8±14.8 in the AMA-M2(+) AIH patients and 324±174 in the PBC patients. The difference was statistically significant ($P=0.0138$).

Comparison of AMA-M2(-) and AMA-M2(+) AIH patients. Next, the laboratory data of the AMA-M2(-) and AMA-M2(+) AIH patients were compared to assess any differences (Table II). AST and ALT levels were higher in the AMA-M2(+) AIH patients than those in the AMA-M2(-) AIH patients, but the difference was not significant.

Follow-up. Three AMA-M2(+) AIH patients were followed-up after liver biopsy (Table III). The levels of AMA-M2, AST, ALT and γ-GTP had decreased in all the patients. Two patients became negative for AMA-M2.

Discussion

It is established that AMA-M2 is more specific to PBC than to AIH (13); therefore, the AMA-M2 titer is a common test for PBC (25,26). A problem with this is that patients with AIH can still test positive for AMA-M2 (21). In a study by Yanagawa *et al* (27), 9 out of 55 patients with AIH were found to be positive for AMA-M2. In the present study, 4 AIH patients

Table II. Comparison between AMA-M2(-) and AMA-M2(+) AIH patients.

Parameter	AMA-M2(-) AIH patients	AMA-M2(+) AIH patients	P-value
Males:females	2:4	0:4	NA
Age (years)	52.3±15.2	59.3±14.8	0.4974
ALP (IU/l)	367±140	457±126	0.3304
AST (IU/l)	198±103	631±212	0.1115
ALT (IU/l)	271±144	663±777	0.2500
γ-GTP (IU/l)	235±116	258±135	0.7800

One-way analysis of variance was applied to the results. AMA-M2, anti-mitochondrial M2 antibody; AIH, autoimmune hepatitis; NA, not applicable; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase.

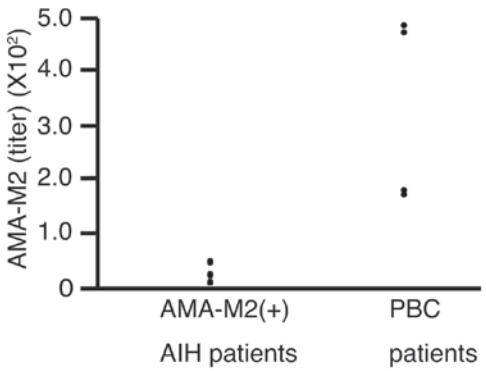

Figure 1. Scatter plot of AMA-M2 titers. Titers of AMA-M2 were 24.8±14.8 (mean ± standard deviation) in the AMA-M2(+) AIH patients and 324±174 in the PBC patients. The difference was statistically significant (P=0.0138, one-way analysis of variance). AMA-M2, anti-mitochondrial M2 antibody; AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis.

were found to be positive for AMA-M2 on admission. The difference in the percentage of patients positive for AMA-M2 may have been dependent on the antigens with which AMA-M2 reacted. The method used by Yanagawa *et al* (27) detected PDC-E2, whereas the method used in the present study detected branched-chain 2-oxo acid dehydrogenase complex and 2-oxoglutaric acid dehydrogenase complex, in addition to PDC-E2. Another reason for the difference may have been the method utilized to detect positivity. Yanagawa *et al* used western blot analysis, while the outsourced laboratory in the present study used a chemiluminescent enzyme immunoassay (CLEIA). The CLEIA is more sensitive than western blot analysis (28).

The titers of AMA-M2 in AIH patients have not been previously reported, to the best of our knowledge. In the present study, the titers of AMA-M2 were significantly lower in the AIH patients than those in the PBC patients, suggesting that a higher AMA-M2 titer is specific to PBC (26). AMA-M2 has been found to persist or disappear after long-term follow-up in AIH patients (29). In the present study, titers of AMA-M2 decreased in the AMA-M2(+) AIH patients.

One major limitation of the present study was that it included a small number of patients. Lower ALP and higher AST/ALT values are characteristics of AIH as compared with PBC (21). The same tendency was found in this study, but

Table III. Follow-up of AMA-M2(+) patients with autoimmune hepatitis.

Parameter	Patient 1	Patient 2	Patient 3
Gender	Female	Female	Female
Age (years)	38	65	72
ALP (IU/l)			
0 months	589	542	531
Follow-up[a]	202	395	158
AST (IU/l)			
0 months	1,460	107	662
Follow-up[a]	14	62	18
ALT (IU/l)			
0 months	1,780	55	585
Follow-up[a]	12	37	15
γ-GTP (IU/l)			
0 months	264	70	387
Follow-up[a]	32	53	65
AMA-M2			
0 months	21.2	9.4	44.9
Follow-up[a]	(-)	(-)	13.1

[a]Follow-up time-points were 50, 38 and 9 months after admission for patients 1, 2 and 3, respectively. ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; AMA-M2: anti-mitochondrial M2 antibody.

the differences were not statistically significant. Despite the small number of patients, the titers of AMA-M2 were found to be significantly higher in the patients with PBC than those in the AMA-M2(+) AIH patients; however, a larger sample of patients is required to confirm these results. Fig. 1 shows a gap in the AMA-M2 titers between the AMA-M2(+) AIH patients and the PBC patients, which indicates that threshold values may exist to differentiate between AMA-M2(+) AIH patients and patients with PBC. A study with a larger sample size is required to investigate this further.

In conclusion, certain patients with AIH were found to be positive for AMA-M2 in the present study, but the titers were

significantly lower than those in the PBC patients. AMA-M2 titers were decreased in the AIH patients at follow-up. Future studies should include a larger sample size and investigate liver biopsies, focusing on bile duct lesions.

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